

(19) World Intellectual Property Organization  
International Bureau(43) International Publication Date  
24 April 2003 (24.04.2003)

PCT

(10) International Publication Number  
WO 03/033658 A2

(51) International Patent Classification<sup>7</sup>: C12N

(21) International Application Number: PCT/US02/32910

(22) International Filing Date: 16 October 2002 (16.10.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/329,508	17 October 2001 (17.10.2001)	US
60/329,747	18 October 2001 (18.10.2001)	US
60/330,835	31 October 2001 (31.10.2001)	US
60/331,478	16 November 2001 (16.11.2001)	US
60/336,726	7 December 2001 (07.12.2001)	US
60/368,548	1 April 2002 (01.04.2002)	US

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(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),

[Continued on next page]

(54) Title: NEUTROKINE-ALPHA AND NEUTROKINE-ALPHA SPLICE VARIANT

Neutrokin- $\alpha$

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1  AAATTCAGGTAACCTCTCTGAGGGGTGAGCCAGCCCTGCATGTAGTGCACGAGAC  60
61  ATCACAACACAGATAACAGGAATGATTCATTCCTGTGTGCTGCTTATCTTAAAGGCC  120
121  CCACCTCTCAAGTTCAGTGTATGATGATGCTCAGCAAGGAGAGCTGACGCG  180
1  MDDSTEREQSRL  12
181  TTACTTCTGCTTAAAGAAAGAGAGAAATGAAGCTGAAGAGTGTGTTCATCTCC  240
13  TSLCLKKREERKLEKECVSILP  32
241  CACGGAAGGAAGCCCTCTGTCGATCTCCAAAGCAGGAAGCTGTGGCTGCAACT  300
33  RKFSPSVRSRKDGKLLAATL  52
361  TGTCTGTGCTGCTGCTGTCTGTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT  360
53  LLAALSSCCLTVVSFYDVAAAL  72
361  TGCAGGGAAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT  420
73  QGDLASLRALQGHAEKLP  92
421  CAGCAGAGAGCAGGAGCCCAAGGCGCTGAGGAGAGCTGAGCTGTACCGCGGAG  480
93  AAGAGAPKAGLEEAFAVTAAGL  112
481  TGAATCTTTGACCAACAGCTCCAGGAGAGCACTCCAGTCAAGACAGCAAGTA  540
113  KIFEPPAPGEGNSSQNSRNK  132
541  AGGTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG  600
133  RAVQGPEETVTFQDCLQLA  152

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A

Neutrokin- $\alpha$

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601  ACAGTGAACACCACTATACAAAAGGATCTTACACATTTGTCATGCTCTCAGCT  660
153  SETPTIQKGSYTFVPKLLSE  172
661  TTAAGGGAAGTGCCTAGAGAAAAGAGATAAATATTGGTCAAGAACTGGTT  720
173  KRGSALEEKENKILVKEETRY  192
721  ACTTTTATATATGCTGAGTTTATATATCTATAGACCTACGCTATGACATCAA  780
193  FFITYGQVLYTDTKYAWGHIL  212
781  TTCAGAGGAAGGTCATGCTTTGGGATGAATTGAGTCTGCTGCTGCTGCTGCT  840
213  QKKVHVFGDELSLVTLFRG  232
841  GTATTCAAAATATGCTGAGACACTACCAATATCTGCTGCTGCTGCTGCTGCTG  900
233  IDNNPEETLPNNSCYSAIAK  252
901  AACTGGAAGAGGAGTGAAGTCCCACTTGCATACCAAGAGAAATGCAAAATAC  960
253  LEEGDELOLAIPRENAQISL  272
961  TGGATGAGATGTCATATTTTGTGCTGATGAGTCTGCTGCTGCTGCTGCTGCT  1020
273  GGDVTFFGALKLL  285
1021  CTGTAGCTATTTTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG  1080
1081  CAAAAA  1100

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B

(57) Abstract: The present invention relates to nucleic acid molecules encoding Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides, including soluble forms of the extracellular domain. Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to antibodies or portions thereof that specifically bind Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV and diagnostic and therapeutic methods using these antibodies. Also provided are diagnostic methods for detecting immune system-related disorders and therapeutic methods for treating immune system-related disorders using the compositions of the invention.



Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,  
ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK,  
TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,  
GW, ML, MR, NE, SN, TD, TG).

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

**Published:**

— *without international search report and to be republished  
upon receipt of that report*

## Neutrokin-alpha and Neutrokin-alpha Splice Variant

### FIELD OF THE INVENTION

[0001] The present invention relates to a novel cytokine which has been designated Neutrokin-alpha ("Neutrokin-alpha"). In addition, an apparent splicing variant of Neutrokin-alpha has been identified and designated Neutrokin-alphaSV. In specific embodiments, the present invention provides nucleic acid molecules encoding Neutrokin-alpha and Neutrokin-alphaSV polypeptides. In additional embodiments, Neutrokin-alpha and Neutrokin-alphaSV polypeptides are also provided, as are vectors, host cells and recombinant methods for producing the same. Neutrokin-alpha is also referred to in the art as TALL-1, THANK, BAFF, zTNF4, TNFSF13B. Neutrokin-alpha is also referred to as the BLyS<sup>TM</sup> protein, from Human Genome Sciences, Inc.

### *Related Art*

[0002] Human tumor necrosis factors (TNF-alpha) and (TNF-beta, or lymphotoxin) are related members of a broad class of polypeptide mediators, which includes the interferons, interleukins and growth factors, collectively called cytokines (Beutler, B. and Cerami, A., *Annu. Rev. Immunol.* 7:625-655 (1989)). Sequence analysis of cytokine receptors has defined several subfamilies of membrane proteins (1) the Ig superfamily, (2) the hematopoietin (cytokine receptor superfamily) and (3) the tumor necrosis factor (TNF)/nerve growth factor (NGF) receptor superfamily (for review of TNF superfamily see, Gruss and Dower, *Blood* 85(12):3378-3404 (1995) and Aggarwal and Natarajan, *Eur. Cytokine Netw.*, 7(2):93-124 (1996)). The TNF/NGF receptor superfamily contains at least 10 different proteins. Gruss and Dower, *supra*. Ligands for these receptors have been identified and belong to at least two cytokine superfamilies. Gruss and Dower, *supra*.

[0003] Tumor necrosis factor (a mixture of TNF-alpha and TNF-beta) was originally discovered as a result of its anti-tumor activity, however, now it is recognized as a pleiotropic cytokine capable of numerous biological activities including apoptosis of some

transformed cell lines, mediation of cell activation and proliferation and also as playing important roles in immune regulation and inflammation.

[0004] To date, known members of the TNF-ligand superfamily include TNF-alpha, TNF-beta (lymphotoxin-alpha), LT-beta, OX40L, Fas ligand, CD30L, CD27L, CD40L and 4-IBBL. The ligands of the TNF ligand superfamily are acidic, TNF-like molecules with approximately 20% sequence homology in the extracellular domains (range, 12%-36%) and exist mainly as membrane-bound forms with the biologically active form being a trimeric/multimeric complex. Soluble forms of the TNF ligand superfamily have only been identified so far for TNF, LT-beta, and Fas ligand (for a general review, see Gruss, H. and Dower, S.K., *Blood*, 85(12) :3378-3404 (1995)), which is hereby incorporated by reference in its entirety. These proteins are involved in regulation of cell proliferation, activation, and differentiation, including control of cell survival or death by apoptosis or cytotoxicity (Armitage, R.J., *Curr. Opin. Immunol.* 6:407 (1994) and Smith, C.A., *Cell* 75:959 (1994)).

[0005] Tumor necrosis factor-alpha (TNF-alpha; also termed cachectin; hereinafter "TNF") is secreted primarily by monocytes and macrophages in response to endotoxin or other stimuli as a soluble homotrimer of 17 kD protein subunits (Smith, R.A. *et al.*, *J. Biol. Chem.* 262:6951-6954 (1987)). A membrane-bound 26 kD precursor form of TNF has also been described (Kriegler, M. *et al.*, *Cell* 53:45-53 (1988)).

[0006] Accumulating evidence indicates that TNF is a regulatory cytokine with pleiotropic biological activities. These activities include: inhibition of lipoprotein lipase synthesis ("cachectin" activity) (Beutler, B. *et al.*, *Nature* 316:552 (1985)), activation of polymorphonuclear leukocytes (Klebanoff, S.J. *et al.*, *J. Immunol.* 136:4220 (1986); Perussia, B., *et al.*, *J. Immunol.* 138:765 (1987)), inhibition of cell growth or stimulation of cell growth (Vilcek, J. *et al.*, *J. Exp. Med.* 163:632 (1986); Sugarman, B. J. *et al.*, *Science* 230:943 (1985); Lachman, L.B. *et al.*, *J. Immunol.* 138:2913 (1987)), cytotoxic action on certain transformed cell types (Lachman, L.B. *et al.*, *supra*; Darzynkiewicz, Z. *et al.*, *Canc. Res.* 44:83 (1984)), antiviral activity (Kohase, M. *et al.*, *Cell* 45:659 (1986); Wong, G.H.W. *et al.*, *Nature* 323:819 (1986)), stimulation of bone resorption (Bertolini, D.R. *et al.*, *Nature* 319:516 (1986); Saklatvala, J., *Nature* 322:547 (1986)), stimulation of collagenase and prostaglandin E2 production (Dayer, J.-M. *et al.*, *J. Exp. Med.* 162:2163 (1985)); and immunoregulatory actions, including activation of T cells (Yokota, S. *et al.*,



*J. Immunol.* 140:531 (1988)), B cells (Kehrl, J.H. *et al.*, *J. Exp. Med.* 166:786 (1987)), monocytes (Philip, R. *et al.*, *Nature* 323:86 (1986)), thymocytes (Ranges, G.E. *et al.*, *J. Exp. Med.* 167:1472 (1988)), and stimulation of the cell-surface expression of major histocompatibility complex (MHC) class I and class II molecules (Collins, T. *et al.*, *Proc. Natl. Acad. Sci. USA* 83:446 (1986); Pujol-Borrel, R. *et al.*, *Nature* 326:304 (1987)).

[0007] TNF is noted for its pro-inflammatory actions which result in tissue injury, such as induction of procoagulant activity on vascular endothelial cells (Poher, J.S. *et al.*, *J. Immunol.* 136:1680 (1986)), increased adherence of neutrophils and lymphocytes (Poher, J.S. *et al.*, *J. Immunol.* 138:3319 (1987)), and stimulation of the release of platelet activating factor from macrophages, neutrophils and vascular endothelial cells (Camussi, G. *et al.*, *J. Exp. Med.* 166:1390 (1987)).

[0008] Recent evidence implicates TNF in the pathogenesis of many infections (Cerami, A. *et al.*, *Immunol. Today* 9:28 (1988)), immune disorders, neoplastic pathology, e.g., in cachexia accompanying some malignancies (Oloff, A. *et al.*, *Cell* 50:555 (1987)), and in autoimmune pathologies and graft-versus host pathology (Piguet, P.-F. *et al.*, *J. Exp. Med.* 166:1280 (1987)). The association of TNF with cancer and infectious pathologies is often related to the host's catabolic state. A major problem in cancer patients is weight loss, usually associated with anorexia. The extensive wasting which results is known as "cachexia" (Kern, K. A. *et al.* *J. Parent. Enter. Nutr.* 12:286-298 (1988)). Cachexia includes progressive weight loss, anorexia, and persistent erosion of body mass in response to a malignant growth. The cachectic state is thus associated with significant morbidity and is responsible for the majority of cancer mortality. A number of studies have suggested that TNF is an important mediator of the cachexia in cancer, infectious pathology, and in other catabolic states.

[0009] TNF is thought to play a central role in the pathophysiological consequences of Gram-negative sepsis and endotoxic shock (Michie, H.R. *et al.*, *Br. J. Surg.* 76:670-671 (1989); Debets, J. M. H. *et al.*, *Second Vienna Shock Forum*, p.463-466 (1989); Simpson, S. Q. *et al.*, *Crit. Care Clin.* 5:27-47 (1989)), including fever, malaise, anorexia, and cachexia. Endotoxin is a potent monocyte/macrophage activator which stimulates production and secretion of TNF (Kornbluth, S.K. *et al.*, *J. Immunol.* 137:2585-2591 (1986)) and other cytokines. Because TNF could mimic many biological effects of endotoxin, it was concluded to be a central mediator responsible for the clinical

manifestations of endotoxin-related illness. TNF and other monocyte-derived cytokines mediate the metabolic and neurohormonal responses to endotoxin (Michie, H.R. *et al.*, *N. Eng. J. Med.* 318:1481-1486 (1988)). Endotoxin administration to human volunteers produces acute illness with flu-like symptoms including fever, tachycardia, increased metabolic rate and stress hormone release (Revhaug, A. *et al.*, *Arch. Surg.* 123:162-170 (1988)). Elevated levels of circulating TNF have also been found in patients suffering from Gram-negative sepsis (Waage, A. *et al.*, *Lancet* 1:355-357 (1987); Hammerle, A.F. *et al.*, *Second Vienna Shock Forum* p. 715-718 (1989); Debets, J. M. H. *et al.*, *Crit. Care Med.* 17:489-497 (1989); Calandra, T. *et al.*, *J. Infec. Dis.* 161:982-987 (1990)).

[0010] Passive immunotherapy directed at neutralizing TNF may have a beneficial effect in Gram-negative sepsis and endotoxemia, based on the increased TNF production and elevated TNF levels in these pathology states, as discussed above. Antibodies to a "modulator" material which was characterized as cachectin (later found to be identical to TNF) were disclosed by Cerami *et al.* (EPO Patent Publication 0,212,489, March 4, 1987). Such antibodies were said to be useful in diagnostic immunoassays and in therapy of shock in bacterial infections. Rubin *et al.* (EPO Patent Publication 0,218,868, April 22, 1987) disclosed monoclonal antibodies to human TNF, the hybridomas secreting such antibodies, methods of producing such antibodies, and the use of such antibodies in immunoassay of TNF. Yone *et al.* (EPO Patent Publication 0,288,088, October 26, 1988) disclosed anti-TNF antibodies, including mAbs, and their utility in immunoassay diagnosis of pathologies, in particular Kawasaki's pathology and bacterial infection. The body fluids of patients with Kawasaki's pathology (infantile acute febrile mucocutaneous lymph node syndrome; Kawasaki, T., *Allergy* 16:178 (1967); Kawasaki, T., *Shonika (Pediatrics)* 26:935 (1985)) were said to contain elevated TNF levels which were related to progress of the pathology (Yone *et al.*, *supra*).

[0011] Other investigators have described mAbs specific for recombinant human TNF which had neutralizing activity *in vitro* (Liang, C-M. *et al.* *Biochem. Biophys. Res. Comm.* 137:847-854 (1986); Meager, A. *et al.*, *Hybridoma* 6:305-311 (1987); Fendly *et al.*, *Hybridoma* 6:359-369 (1987); Bringman, T S *et al.*, *Hybridoma* 6:489-507 (1987); Hirai, M. *et al.*, *J. Immunol. Meth.* 96:57-62 (1987); Moller, A. *et al.* (*Cytokine* 2:162-169 (1990)). Some of these mAbs were used to map epitopes of human TNF and develop enzyme immunoassays (Fendly *et al.*, *supra*; Hirai *et al.*, *supra*; Moller *et al.*, *supra*) and

to assist in the purification of recombinant TNF (Bringman *et al.*, *supra*). However, these studies do not provide a basis for producing TNF neutralizing antibodies that can be used for *in vivo* diagnostic or therapeutic uses in humans, due to immunogenicity, lack of specificity and/or pharmaceutical suitability.

[0012] Neutralizing antisera or mAbs to TNF have been shown in mammals other than man to abrogate adverse physiological changes and prevent death after lethal challenge in experimental endotoxemia and bacteremia. This effect has been demonstrated, e.g., in rodent lethality assays and in primate pathology model systems (Mathison, J.C. *et al.*, *J. Clin. Invest.* 81:1925-1937 (1988); Beutler, B. *et al.*, *Science* 229:869-871 (1985); Tracey, K. J. *et al.*, *Nature* 330:662-664 (1987); Shimamoto, Y. *et al.*, *Immunol. Lett.* 17:311-318 (1988); Silva, A. T. *et al.*, *J. Infect. Dis.* 162:421-427 (1990); Opal, S. M. *et al.*, *J. Infect. Dis.* 161:1148-1152 (1990); Hinshaw, L.B. *et al.*, *Circ. Shock* 30:279-292 (1990)).

[0013] To date, experience with anti-TNF mAb therapy in humans has been limited but shows beneficial therapeutic results, e.g., in arthritis and sepsis. See, e.g., Elliott, M. J. *et al.*, *Baillieres Clin. Rheumatol.* 9:633-52 (1995); Feldmann M, *et al.*, *Ann. N. Y. Acad. Sci. USA* 766:272-8 (1995); van der Poll, T. *et al.*, *Shock* 3:1-12 (1995); Wherry *et al.*, *Crit. Care. Med.* 21:S436-40 (1993); Tracey K. J., *et al.*, *Crit. Care Med.* 21:S415-22 (1993).

[0014] Mammalian development is dependent on both the proliferation and differentiation of cells as well as programmed cell death which occurs through apoptosis (Walker, *et al.*, *Methods Achiev. Exp. Pathol.* 13:18 (1988). Apoptosis plays a critical role in the destruction of immune thymocytes that recognize self antigens. Failure of this normal elimination process may play a role in autoimmune diseases (Gammon *et al.*, *Immunology Today* 12:193 (1991)).

[0015] Itoh *et al.* (*Cell* 66:233 (1991)) described a cell surface antigen, Fas/CD95 that mediates apoptosis and is involved in clonal deletion of T-cells. Fas is expressed in activated T-cells, B-cells, neutrophils and in thymus, liver, heart and lung and ovary in adult mice (Watanabe-Fukunaga *et al.*, *J. Immunol.* 148:1274 (1992)) in addition to activated T-cells, B-cells, neutrophils. In experiments where a monoclonal Ab is cross-linked to Fas, apoptosis is induced (Yonehara *et al.*, *J. Exp. Med.* 169:1747 (1989); Trauth *et al.*, *Science* 245:301 (1989)). In addition, there is an example where binding of a

monoclonal Ab to Fas is stimulatory to T-cells under certain conditions (Alderson *et al.*, *J. Exp. Med.* 178:2231 (1993)).

[0016] Fas antigen is a cell surface protein of relative MW of 45 Kd. Both human and murine genes for Fas have been cloned by Watanabe-Fukunaga *et al.*, (*J. Immunol.* 148:1274 (1992)) and Itoh *et al.* (*Cell* 66:233 (1991)). The proteins encoded by these genes are both transmembrane proteins with structural homology to the Nerve Growth Factor/Tumor Necrosis Factor receptor superfamily, which includes two TNF receptors, the low affinity Nerve Growth Factor receptor and CD40, CD27, CD30, and OX40.

[0017] Recently the Fas ligand has been described (Suda *et al.*, *Cell* 75:1169 (1993)). The amino acid sequence indicates that Fas ligand is a type II transmembrane protein belonging to the TNF family. Thus, the Fas ligand polypeptide comprises three main domains: a short intracellular domain at the amino terminal end and a longer extracellular domain at the carboxy terminal end, connected by a hydrophobic transmembrane domain. Fas ligand is expressed in splenocytes and thymocytes, consistent with T-cell mediated cytotoxicity. The purified Fas ligand has a MW of 40 kD.

[0018] Recently, it has been demonstrated that Fas/Fas ligand interactions are required for apoptosis following the activation of T-cells (Ju *et al.*, *Nature* 373:444 (1995); Brunner *et al.*, *Nature* 373:441 (1995)). Activation of T-cells induces both proteins on the cell surface. Subsequent interaction between the ligand and receptor results in apoptosis of the cells. This supports the possible regulatory role for apoptosis induced by Fas/Fas ligand interaction during normal immune responses.

[0019] Accordingly, there is a need to provide cytokines similar to TNF that are involved in pathological conditions. Such novel cytokines may be used to make novel antibodies or other antagonists that bind these TNF-like cytokines for diagnosis and therapy of disorders related to TNF-like cytokines.

### ***Summary of the Invention***

[0020] In accordance with one embodiment of the present invention, there is provided a novel extracellular domain of a Neutrokin-alpha polypeptide, and a novel extracellular domain of a Neutrokin-alphaSV polypeptide, as well as biologically active and diagnostically or therapeutically useful fragments, analogs and derivatives thereof.

**[0021]** In accordance with another embodiment of the present invention, there are provided isolated nucleic acid molecules encoding human Neutrokin-alpha or Neutrokin-alphaSV, including mRNAs, DNAs, cDNAs, genomic DNAs as well as analogs and biologically active and diagnostically or therapeutically useful fragments and derivatives thereof.

**[0022]** The present invention provides isolated nucleic acid molecules comprising, or alternatively, consisting of, a polynucleotide encoding a cytokine and an apparent splice variant thereof that are structurally similar to TNF and related cytokines and have similar biological effects and activities. This cytokine is named Neutrokin-alpha and the invention includes Neutrokin-alpha polypeptides having at least a portion of the amino acid sequence in Figures 1A and 1B (SEQ ID NO:2) or amino acid sequence encoded by the cDNA clone (HNEDU15) deposited on October 22, 1996 assigned ATCC number 97768. The nucleotide sequence determined by sequencing the deposited Neutrokin-alpha clone, which is shown in Figures 1A and 1B (SEQ ID NO:1), contains an open reading frame encoding a complete polypeptide of 285 amino acid residues including an N-terminal methionine, a predicted intracellular domain of about 46 amino acid residues, a predicted transmembrane domain of about 26 amino acids, a predicted extracellular domain of about 213 amino acids, and a deduced molecular weight for the complete protein of about 31 kDa. As for other type II transmembrane proteins, soluble forms of Neutrokin-alpha include all or a portion of the extracellular domain cleaved from the transmembrane domain and a polypeptide comprising the complete Neutrokin-alpha polypeptide lacking the transmembrane domain, i.e., the extracellular domain linked to the intracellular domain. The apparent splice variant of Neutrokin-alpha is named Neutrokin-alphaSV and the invention includes Neutrokin-alphaSV polypeptides comprising, or alternatively, consisting of, at least a portion of the amino acid sequence in Figures 5A and 5B (SEQ ID NO:19) or amino acid sequence encoded by the cDNA clone HDPMC52 deposited on December 10, 1998 and assigned ATCC number 203518. The nucleotide sequence determined by sequencing the deposited Neutrokin-alphaSV clone, which is shown in Figures 5A and 5B (SEQ ID NO:18), contains an open reading frame encoding a complete polypeptide of 266 amino acid residues including an N-terminal methionine, a predicted intracellular domain of about 46 amino acid residues, a predicted transmembrane domain of about 26 amino acids, a

predicted extracellular domain of about 194 amino acids, and a deduced molecular weight for the complete protein of about 29 kDa. As for other type II transmembrane proteins, soluble forms of Neutrokin- $\alpha$ SV include all or a portion of the extracellular domain cleaved from the transmembrane domain and a polypeptide comprising the complete Neutrokin- $\alpha$ SV polypeptide lacking the transmembrane domain, i.e., the extracellular domain linked to the intracellular domain.

**[0023]** Thus, one embodiment of the invention provides an isolated nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide having a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding a full-length Neutrokin- $\alpha$  polypeptide having the complete amino acid sequence in Figures 1A and 1B (SEQ ID NO:2) or as encoded by the cDNA clone contained in the deposit having ATCC accession number 97768; (b) a nucleotide sequence encoding the predicted extracellular domain of the Neutrokin- $\alpha$  polypeptide having the amino acid sequence at positions 73 to 285 in Figures 1A and 1B (SEQ ID NO:2) or as encoded by the clone contained in the deposit having ATCC accession number 97768; (c) a nucleotide sequence encoding a fragment of the polypeptide of (b) (e.g., amino acids 134-285) having Neutrokin- $\alpha$  functional activity (e.g., biological activity); (d) a nucleotide sequence encoding a polypeptide comprising the Neutrokin- $\alpha$  intracellular domain (predicted to constitute amino acid residues from about 1 to about 46 in Figures 1A and 1B (SEQ ID NO:2)) or as encoded by the clone contained in the deposit having ATCC accession number 97768; (e) a nucleotide sequence encoding a polypeptide comprising the Neutrokin- $\alpha$  transmembrane domain (predicted to constitute amino acid residues from about 47 to about 72 in Figures 1A and 1B (SEQ ID NO:2) or as encoded by the cDNA clone contained in the deposit having ATCC accession number 97768; (f) a nucleotide sequence encoding a soluble Neutrokin- $\alpha$  polypeptide having the extracellular and intracellular domains but lacking the transmembrane domain; and (g) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e) or (f) above.

**[0024]** Further embodiments of the invention include isolated nucleic acid molecules that comprise, or alternatively consist of, a polynucleotide having a nucleotide sequence at least 80%, 85% or 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical, to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f) or (g) above,

or a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide in (a), (b), (c), (d), (e), (f) or (g) above. This polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues.

**[0025]** Another embodiment of the invention provides an isolated nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide having a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding a full-length Neutrokine-alphaSV polypeptide having the complete amino acid sequence in Figures 5A and 5B (SEQ ID NO:19) or as encoded by the cDNA clone contained in the ATCC Deposit deposited on December 10, 1998 as ATCC Number 203518; (b) a nucleotide sequence encoding the predicted extracellular domain of the Neutrokine-alphaSV polypeptide having the amino acid sequence at positions 73 to 266 in Figures 1A and 1B (SEQ ID NO:2) or as encoded by the cDNA clone contained in ATCC 203518 deposited on December 10, 1998; (c) a nucleotide sequence encoding a polypeptide comprising the Neutrokine-alphaSV intracellular domain (predicted to constitute amino acid residues from about 1 to about 46 in Figures 5A and 5B (SEQ ID NO:19)) or as encoded by the cDNA clone contained in ATCC No. 203518 deposited on December 10, 1998; (d) a nucleotide sequence encoding a polypeptide comprising the Neutrokine-alphaSV transmembrane domain (predicted to constitute amino acid residues from about 47 to about 72 in Figures 5A and 5B (SEQ ID NO:19) or as encoded by the cDNA clone contained in ATCC No. 203518 deposited on December 10, 1998; (e) a nucleotide sequence encoding a soluble Neutrokine-alphaSV polypeptide having the extracellular and intracellular domains but lacking the transmembrane domain; and (f) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), or (e) above.

**[0026]** Further embodiments of the invention include isolated nucleic acid molecules that comprise, or alternatively consist of, a polynucleotide having a nucleotide sequence at least 80%, 85% or 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical, to any of the nucleotide sequences in (a), (b), (c), (d), (e) or (f) above, or a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide in (a), (b), (c), (d), (e) or (f) above. This polynucleotide which hybridizes

does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues.

**[0027]** In one embodiment, the invention includes isolated nucleic acid molecules that comprise, or alternatively consist of, a polynucleotide having a nucleotide sequence encoding the apparent splice variant of Neutrokin- $\alpha$  comprising, or alternatively consisting of, at least a portion of the amino acid sequence from Gly-142 to Leu-266 as shown in Figures 5A and 5B (SEQ ID NO:19) or amino acid sequence encoded by the cDNA clone HDPMC52 deposited on December 10, 1998 and assigned ATCC Deposit No. 203518.

**[0028]** In another preferred embodiment, the invention include isolated nucleic acid molecules that comprise, or alternatively consist of, a polynucleotide having a nucleotide sequence encoding the apparent splice variant of Neutrokin- $\alpha$  comprising, or alternatively consisting of, at least a portion of the amino acid sequence from Ala-134 to Leu-266 as shown in Figures 5A and 5B (SEQ ID NO:19) or amino acid sequence encoded by the cDNA clone HDPMC52 deposited on December 10, 1998 and assigned ATCC Deposit No. 203518.

**[0029]** In additional embodiments, the nucleic acid molecules of the invention comprise, or alternatively consist of, a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of a Neutrokin- $\alpha$  or Neutrokin- $\alpha$ SV polypeptide having an amino acid sequence in (a), (b), (c), (d), (e), (f) or (g) above. A further nucleic acid embodiment of the invention relates to an isolated nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide which encodes the amino acid sequence of a Neutrokin- $\alpha$  or Neutrokin- $\alpha$ SV polypeptide having an amino acid sequence which contains at least one amino acid addition, substitution, and/or deletion but not more than 50 amino acid additions, substitutions and/or deletions, even more preferably, not more than 40 amino acid additions, substitutions, and/or deletions, still more preferably, not more than 30 amino acid additions, substitutions, and/or deletions, and still even more preferably, not more than 20 amino acid additions, substitutions, and/or deletions. Of course, in order of ever-increasing preference, it is highly preferable for a polynucleotide which encodes the amino acid sequence of a Neutrokin- $\alpha$  or Neutrokin- $\alpha$ SV polypeptide to have an amino acid sequence which contains not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 or 1-100, 1-50, 1-25, 1-20, 1-15,



1-10, or 1-5 amino acid additions, substitutions and/or deletions. Conservative substitutions are preferable.

**[0030]** The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells and for using them for production of Neutrokin- $\alpha$  polypeptides by recombinant techniques.

**[0031]** In accordance with a further embodiment of the present invention, there is provided a process for producing such polypeptides by recombinant techniques comprising culturing recombinant prokaryotic and/or eukaryotic host cells, containing a Neutrokin- $\alpha$  or Neutrokin- $\alpha$ SV nucleic acid sequence of the invention, under conditions promoting expression of said polypeptide and subsequent recovery of said polypeptide.

**[0032]** The invention further provides an isolated Neutrokin- $\alpha$  polypeptide comprising, or alternatively consisting of, an amino acid sequence selected from the group consisting of: (a) the amino acid sequence of the full-length Neutrokin- $\alpha$  polypeptide having the complete amino acid sequence shown in Figures 1A and 1B (i.e., positions 1-285 of SEQ ID NO:2) or as encoded by the cDNA plasmid contained in the deposit having ATCC accession number 97768; (b) the amino acid sequence of the full-length Neutrokin- $\alpha$  polypeptide having the complete amino acid sequence shown in SEQ ID NO:2 excepting the N-terminal methionine (i.e., positions 2 to 285 of SEQ ID NO:2); (c) a fragment of the polypeptide of (b) having Neutrokin- $\alpha$  functional activity (e.g., biological activity); (d) the amino acid sequence of the predicted extracellular domain of the Neutrokin- $\alpha$  polypeptide having the amino acid sequence at positions 73 to 285 in Figures 1A and 1B (SEQ ID NO:2) or as encoded by the cDNA plasmid contained in the deposit having ATCC accession number 97768; (e) an amino acid sequence encoding the mature soluble form of Neutrokin- $\alpha$  polypeptide having the amino acid sequence at positions 134-285 in Figures 1A and 1B (SEQ ID NO:2); (f) the amino acid sequence of the Neutrokin- $\alpha$  intracellular domain (predicted to constitute amino acid residues from about 1 to about 46 in Figures 1A and 1B (SEQ ID NO:2)) or as encoded by the cDNA plasmid contained in the deposit having ATCC accession number 97768; (g) the amino acid sequence of the Neutrokin- $\alpha$  transmembrane domain (predicted to constitute amino acid residues from about 47 to about 72 in Figures 1A and 1B (SEQ ID

NO:2)) or as encoded by the cDNA plasmid contained in the deposit having ATCC accession number 97768; (h) the amino acid sequence of the soluble Neutrokin- $\alpha$  polypeptide having the extracellular and intracellular domains but lacking the transmembrane domain, wherein each of these domains is defined above; and (i) fragments of the polypeptide of (a), (b), (c), (d), (e), (f), (g) or (h). The polypeptides of the present invention also include polypeptides having an amino acid sequence at least 80% identical, more preferably at least 85% or 90% identical, and still more preferably 95%, 96%, 97%, 98% or 99% identical to those described in (a), (b), (c), (d), (e) (f), (g), (h) or (i) above, as well as polypeptides having an amino acid sequence with at least 80%, 85%, or 90% similarity, and more preferably at least 95% similarity, to those above. Additional embodiments of the invention relates to polypeptides which comprise, or alternatively consist of, the amino acid sequence of an epitope-bearing portion of a Neutrokin- $\alpha$  polypeptide having an amino acid sequence described in (a), (b), (c), (d), (e), (f), (g), (h) or (i) above. Polypeptides having the amino acid sequence of an epitope-bearing portion of a Neutrokin- $\alpha$  polypeptide of the invention include portions of such polypeptides with at least 4, at least 5, at least 6, at least 7, at least 8, and preferably at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and more preferably at least about 30 amino acids to about 50 amino acids, although epitope-bearing polypeptides of any length up to and including the entire amino acid sequence of a polypeptide of the invention described above also are included in the invention.

**[0033]** Highly preferred embodiments of the invention are directed to nucleic acid molecules comprising, or alternatively consisting of a polynucleotide having a nucleotide sequence at least 80%, 85%, 90% identical and more preferably at least 95%, 96%, 97%, 98%, 99% or 100% identical to a polynucleotide sequence encoding the Neutrokin- $\alpha$  polypeptide having the amino acid sequence at positions 134-285 in Figures 1A and 1B (SEQ ID NO:2). Preferred embodiments of the invention are directed to nucleic acid molecules comprising, or alternatively consisting of a polynucleotide having a nucleotide sequence at least 90% identical to a polynucleotide sequence encoding the Neutrokin- $\alpha$  polypeptide having the amino acid sequence at positions 134-285 in Figures 1A and 1B (SEQ ID NO:2). More preferred embodiments of the invention are directed to nucleic acid molecules comprising, or alternatively consisting of a polynucleotide having a

nucleotide sequence at least 95% identical to a polynucleotide sequence encoding the Neutrokin- $\alpha$  polypeptide having the amino acid sequence at positions 134-285 in Figures 1A and 1B (SEQ ID NO:2). More preferred embodiments of the invention are directed to nucleic acid molecules comprising, or alternatively consisting of a polynucleotide having a nucleotide sequence at least 96% identical to a polynucleotide sequence encoding the Neutrokin- $\alpha$  polypeptide having the amino acid sequence at positions 134-285 in Figures 1A and 1B (SEQ ID NO:2). Additionally, more preferred embodiments of the invention are directed to nucleic acid molecules comprising, or alternatively consisting of a polynucleotide having a nucleotide sequence at least 97% to a polynucleotide sequence encoding the Neutrokin- $\alpha$  polypeptide having the amino acid sequence at positions 134-285 in Figures 1A and 1B (SEQ ID NO:2). Additionally, more preferred embodiments of the invention are directed to nucleic acid molecules comprising, or alternatively consisting of a polynucleotide having a nucleotide sequence at least 98% to a polynucleotide sequence encoding the Neutrokin- $\alpha$  polypeptide having the amino acid sequence at positions 134-285 in Figures 1A and 1B (SEQ ID NO:2). Additionally, more preferred embodiments of the invention are directed to nucleic acid molecules comprising, or alternatively consisting of a polynucleotide having a nucleotide sequence at least 99% identical to a polynucleotide sequence encoding the Neutrokin- $\alpha$  polypeptide having the amino acid sequence at positions 134-285 in Figures 1A and 1B (SEQ ID NO:2).

**[0034]** The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these polynucleotides and nucleic acid molecules are also encompassed by the invention.

**[0035]** The invention further provides an isolated Neutrokin- $\alpha$ SV polypeptide comprising, or alternatively consisting of, an amino acid sequence selected from the group consisting of: (a) the amino acid sequence of the full-length Neutrokin- $\alpha$ SV polypeptide having the complete amino acid sequence shown in Figures 5A and 5B (i.e., positions 1-266 of SEQ ID NO:19) or as encoded by the cDNA clone contained in ATCC No. 203518 deposited on December 10, 1998; (b) the amino acid sequence of the full-length Neutrokin- $\alpha$ SV polypeptide having the complete amino acid sequence shown in SEQ ID NO:19 excepting the N-terminal methionine (i.e., positions 2 to 266 of SEQ ID NO:19); (c) the amino acid sequence of the predicted extracellular domain of the

Neutrokinine-alphaSV polypeptide having the amino acid sequence at positions 73 to 266 in Figures 5A and 5B (SEQ ID NO:19) or as encoded by the cDNA clone contained in ATCC No. 203518 deposited on December 10, 1998; (d) the amino acid sequence of the Neutrokinine-alphaSV intracellular domain (predicted to constitute amino acid residues from about 1 to about 46 in Figures 5A and 5B (SEQ ID NO:19)) or as encoded by the cDNA clone contained in ATCC No. 203518 deposited on December 10, 1998; (e) the amino acid sequence of the Neutrokinine-alphaSV transmembrane domain (predicted to constitute amino acid residues from about 47 to about 72 in Figures 5A and 5B (SEQ ID NO:19)) or as encoded by the cDNA clone contained in ATCC No. 203518 deposited on December 10, 1998; (f) the amino acid sequence of the soluble Neutrokinine-alphaSV polypeptide having the extracellular and intracellular domains but lacking the transmembrane domain, wherein each of these domains is defined above; and (g) fragments of the polypeptide of (a), (b), (c), (d), (e), or (f). The polypeptides of the present invention also include polypeptides having an amino acid sequence at least 80% identical, more preferably at least 85% or 90% identical, and still more preferably 95%, 96%, 97%, 98% or 99% identical to those described in (a), (b), (c), (d), (e) (f), or (g) above, as well as polypeptides having an amino acid sequence with at least 80%, 85%, or 90% similarity, and more preferably at least 95% similarity, to those above. Additional embodiments of the invention relates to polypeptides which comprise, or alternatively consist of, the amino acid sequence of an epitope-bearing portion of a Neutrokinine-alphaSV polypeptide having an amino acid sequence described in (a), (b), (c), (d), (e), (f), or (g) above. Peptides or polypeptides having the amino acid sequence of an epitope-bearing portion of a Neutrokinine-alphaSV polypeptide of the invention include portions of such polypeptides with at least 4, at least 5, at least 6, at least 7, at least 8, and preferably at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and more preferably at least about 30 amino acids to about 50 amino acids, although epitope-bearing polypeptides of any length up to and including the entire amino acid sequence of a polypeptide of the invention described above also are included in the invention.

[0036] Certain non-exclusive embodiments of the invention relate to a polypeptide which has the amino acid sequence of an epitope-bearing portion of a Neutrokinine-alpha or Neutrokinine-alphaSV polypeptide having an amino acid sequence described in (a), (b), (c),

(d), (e), (f), (g), (h) or (i) above. In other embodiments, the invention provides an isolated antibody that binds specifically (i.e., uniquely) to a Neutrokin-alpha or Neutrokin-alphaSV polypeptide having an amino acid sequence described in (a), (b), (c), (d), (e), (f), (g), (h) or (i) above.

**[0037]** The invention further provides methods for isolating antibodies that bind specifically (i.e., uniquely) to a Neutrokin-alpha or Neutrokin-alphaSV polypeptide having an amino acid sequence as described herein. Such antibodies are useful diagnostically or therapeutically as described below.

**[0038]** The invention also provides for pharmaceutical compositions comprising soluble Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides, particularly human Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides, and/or anti-Neutrokin-alpha antibodies and/or anti-Neutrokin-alphaSV antibodies which may be employed, for instance, to treat, prevent, prognose and/or diagnose tumor and tumor metastasis, infections by bacteria, viruses and other parasites, immunodeficiencies, inflammatory diseases, lymphadenopathy, autoimmune diseases, graft versus host disease, stimulate peripheral tolerance, destroy some transformed cell lines, mediate cell activation, survival and proliferation, mediate immune regulation and inflammatory responses, and to enhance or inhibit immune responses.

**[0039]** In certain embodiments, soluble Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides of the invention, or agonists thereof, are administered, to treat, prevent, prognose and/or diagnose an immunodeficiency (e.g., severe combined immunodeficiency (SCID)-X linked, SCID-autosomal, adenosine deaminase deficiency (ADA deficiency), X-linked agammaglobulinemia (XLA), Bruton's disease, congenital agammaglobulinemia, X-linked infantile agammaglobulinemia, acquired agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, transient hypogammaglobulinemia of infancy, unspecified hypogammaglobulinemia, agammaglobulinemia, common variable immunodeficiency (CVID) (acquired), Wiskott-Aldrich Syndrome (WAS), X-linked immunodeficiency with hyper IgM, non X-linked immunodeficiency with hyper IgM, selective IgA deficiency, IgG subclass deficiency (with or without IgA deficiency), antibody deficiency with normal or elevated Igs, immunodeficiency with thymoma, Ig heavy chain deletions, kappa chain deficiency, B cell lymphoproliferative disorder

(BLPD), selective IgM immunodeficiency, recessive agammaglobulinemia (Swiss type), reticular dysgenesis, neonatal neutropenia, severe congenital leukopenia, thymic aplasia-aplasia or dysplasia with immunodeficiency, ataxia-telangiectasia, short limbed dwarfism, X-linked lymphoproliferative syndrome (XLP), Nezelof syndrome-combined immunodeficiency with Igs, purine nucleoside phosphorylase deficiency (PNP), MHC Class II deficiency (Bare Lymphocyte Syndrome) and severe combined immunodeficiency.) or conditions associated with an immunodeficiency.

**[0040]** In a specific embodiment, Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat, prevent, prognose and/or diagnose common variable immunodeficiency.

**[0041]** In a specific embodiment, Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat, prevent, prognose and/or diagnose X-linked agammaglobulinemia.

**[0042]** In another specific embodiment, Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat, prevent, prognose and/or diagnose severe combined immunodeficiency (SCID).

**[0043]** In another specific embodiment, Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat, prevent, prognose and/or diagnose Wiskott-Aldrich syndrome.

**[0044]** In another specific embodiment, Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat, prevent, prognose and/or diagnose X-linked Ig deficiency with hyper IgM.

**[0045]** In another embodiment, Neutrokin- $\alpha$  antagonists and/or Neutrokin- $\alpha$ SV antagonists (e.g., an anti-Neutrokin- $\alpha$  antibody), are administered to treat, prevent, prognose and/or diagnose an autoimmune disease (e.g., rheumatoid arthritis, systemic lupus erythematosus, idiopathic thrombocytopenia purpura, autoimmune hemolytic anemia, autoimmune neonatal thrombocytopenia, autoimmunocytopenia, hemolytic anemia, antiphospholipid syndrome, dermatitis, allergic encephalomyelitis, myocarditis, relapsing polychondritis, rheumatic heart disease, glomerulonephritis (e.g., IgA nephropathy), an immune-based rheumatologic disease (e.g., SLE, rheumatoid arthritis, CREST syndrome (a variant of scleroderma characterized by calcinosis, Raynaud's phenomenon, esophageal motility disorders, sclerodactyly, and telangiectasia.),

Seronegative spondyloarthropathy (SpA), Polymyositis/dermatomyositis, Microscopic polyangiitis, Hepatitis C-associated arthritis, Takayasu's arteritis, and undifferentiated connective tissue disorder), Multiple Sclerosis, Neuritis, Uveitis Ophthalmia, Polyendocrinopathies, Purpura (e.g., Henloch-Schoenlein purpura), Reiter's Disease, Stiff-Man Syndrome, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye, autoimmune thyroiditis, hypothyroidism (i.e., Hashimoto's thyroiditis, Goodpasture's syndrome, Pemphigus, Receptor autoimmunities such as, for example, (a) Graves' Disease , (b) Myasthenia Gravis, and (c) insulin resistance, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura , scleroderma with anti-collagen antibodies, mixed connective tissue disease, polymyositis/dermatomyositis, pernicious anemia, idiopathic Addison's disease, infertility, glomerulonephritis such as primary glomerulonephritis and IgA nephropathy, bullous pemphigoid, Sjogren's syndrome, diabetes mellitus, and adrenergic drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis), chronic active hepatitis, primary biliary cirrhosis, other endocrine gland failure, vitiligo, vasculitis, post-MI, cardiomyopathy syndrome, urticaria, atopic dermatitis, asthma, inflammatory myopathies, and other inflammatory, granulomatous, degenerative, and atrophic disorders) or conditions associated with an autoimmune disease. In a specific preferred embodiment, rheumatoid arthritis is treated, prevented, prognosed and/or diagnosed using anti-Neurokinine-alpha antibodies and/or anti-Neurokinine-alphaSV antibodies and/or other antagonist of the invention. In another specific preferred embodiment, systemic lupus erythematosus is treated, prevented, prognosed, and/or diagnosed using anti-Neurokinine-alpha antibodies and/or anti-Neurokinine-alphaSV and/or other antagonist of the invention. In another specific preferred embodiment, idiopathic thrombocytopenia purpura is treated, prevented, prognosed, and/or diagnosed using anti-Neurokinine-alpha antibodies and/or anti-Neurokinine-alphaSV and/or other antagonist of the invention. In another specific preferred embodiment IgA nephropathy is treated, prevented, prognosed and/or diagnosed using anti-Neurokinine-alpha antibodies and/or anti-Neurokinine-alphaSV and/or other antagonist of the invention. In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, prognosed and/or diagnosed using anti-Neurokinine-alpha antibodies and/or anti-Neurokinine-alphaSV antibodies.

[0046] The invention further provides compositions comprising a Neutrokin- $\alpha$  or Neutrokin- $\alpha$ SV polynucleotide, a Neutrokin- $\alpha$  or Neutrokin- $\alpha$ SV polypeptide, and/or an anti-Neutrokin- $\alpha$  antibody or anti-Neutrokin- $\alpha$ SV antibody, for administration to cells *in vitro*, to cells *ex vivo*, and to cells *in vivo*, or to a multicellular organism. In preferred embodiments, the compositions of the invention comprise a Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polynucleotide for expression of a Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide in a host organism for treatment of disease. In a most preferred embodiment, the compositions of the invention comprise a Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polynucleotide for expression of a Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide in a host organism for treatment of an immunodeficiency and/or conditions associated with an immunodeficiency. Particularly preferred in this regard is expression in a human patient for treatment of a dysfunction associated with aberrant endogenous activity of a Neutrokin- $\alpha$ , Neutrokin- $\alpha$ SV, Neutrokin  $\alpha$  receptor, and/or Neutrokin- $\alpha$ SV receptor gene (e.g., expression to enhance the normal B-cell function by expanding B-cell numbers or increasing B cell lifespan).

[0047] The present invention further encompasses methods and compositions for preventing, treating and/or ameliorating diseases or disorders associated with aberrant or inappropriate Neutrokin- $\alpha$ , Neutrokin- $\alpha$ SV, Neutrokin- $\alpha$  receptor, and/or Neutrokin- $\alpha$ SV receptor expression or function in an animal, preferably a mammal, and most preferably a human, comprising, or alternatively consisting of, administering to an animal in which such treatment, prevention or amelioration is desired one or more Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides (including molecules which comprise, or alternatively consist of, Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide fragments or variants thereof) in an amount effective to treat prevent or ameliorate the disease or disorder.

[0048] The present invention further encompasses methods and compositions for killing cells of hematopoietic origin, comprising, or alternatively consisting of, contacting Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide with cells of hematopoietic origin. In preferred embodiments, the cells of hematopoietic origin are B cells.

[0049] The present invention further encompasses methods and compositions for killing cells of hematopoietic origin, comprising, or alternatively consisting of,



administering to an animal in which such killing is desired, a Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide (e.g., a radiolabeled Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide) in an amount effective to kill cells of hematopoietic origin. In preferred embodiments, the cells of hematopoietic origin are B cells.

**[0050]** The present invention further encompasses methods and compositions for stimulating immunoglobulin production, comprising, or alternatively consisting of, contacting an effective amount of Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV with cells of hematopoietic origin, wherein the effective amount of the Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV binding polypeptide stimulates Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV-mediated immunoglobulin production.

**[0051]** The present invention further encompasses methods and compositions for stimulating immunoglobulin production comprising, or alternatively consisting of, administering to an animal in which such stimulation is desired, a Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide in an amount effective to stimulate immunoglobulin production.

**[0052]** The present invention further encompasses methods and compositions for stimulating proliferation of cells of hematopoietic origin, comprising, or alternatively consisting of, contacting an effective amount of Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide with cells of hematopoietic origin, wherein the effective amount of Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide stimulates Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV-mediated cell proliferation. In preferred embodiments, the cells of hematopoietic origin are B cells.

**[0053]** The present invention further encompasses methods and compositions for stimulating proliferation of cells of hematopoietic origin, comprising, or alternatively consisting of, administering to an animal in which such stimulation is desired, a Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide in an amount effective to stimulate Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV-mediated cell proliferation. In preferred embodiments, the cells of hematopoietic origin are B cells.

**[0054]** The present invention further encompasses methods and compositions for increasing activation of cells of hematopoietic origin, comprising, or alternatively consisting of, contacting an effective amount of Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide with cells of hematopoietic origin, wherein the effective amount of

Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide increases Neutrokin-alpha and/or Neutrokin-alphaSV-mediated activation of cells of hematopoietic origin. In preferred embodiments, the cells of hematopoietic origin are B cells.

[0055] The present invention further encompasses methods and compositions for increasing activation of cells of hematopoietic origin, comprising, or alternatively consisting of, administering to an animal in which such increase is desired, a Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide in an amount effective to increase Neutrokin-alpha and/or Neutrokin-alphaSV-mediated activation of cells of hematopoietic origin. In preferred embodiments, the cells of hematopoietic origin are B cells.

[0056] The present invention further encompasses methods and compositions for increasing lifespan of cells of hematopoietic origin, comprising, or alternatively consisting of, contacting an effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV binding polypeptide increases Neutrokin-alpha and/or Neutrokin-alphaSV-regulated lifespan of cells of hematopoietic origin. In preferred embodiments, the cells of hematopoietic origin are B cells.

[0057] The present invention further encompasses methods and compositions for increasing lifespan of cells of hematopoietic origin, comprising, or alternatively consisting of, administering to an animal in which such increase is desired, a Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide in an amount effective to increase Neutrokin-alpha and/or Neutrokin-alphaSV-regulated lifespan of cells of hematopoietic origin. In preferred embodiments, the cells of hematopoietic origin are B cells.

[0058] The present invention further encompasses methods and compositions for inhibiting or reducing immunoglobulin production, comprising, or alternatively consisting of, contacting an effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV with cells of hematopoietic origin, wherein the effective amount of the Neutrokin-alpha and/or Neutrokin-alphaSV binding polypeptide inhibits or reduces Neutrokin-alpha and/or Neutrokin-alphaSV-mediated immunoglobulin production. In preferred embodiments, the cells of hematopoietic origin are B cells.

[0059] The present invention further encompasses methods and compositions for inhibiting or reducing immunoglobulin production comprising, or alternatively consisting

of, administering to an animal in which such inhibition or reduction is desired, a Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide in an amount effective to inhibit or reduce immunoglobulin production.

**[0060]** The present invention further encompasses methods and compositions for inhibiting or reducing proliferation of cells of hematopoietic origin, comprising, or alternatively consisting of, contacting an effective amount of Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide with cells of hematopoietic origin, wherein the effective amount of Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide inhibits or reduces Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV-mediated cell proliferation. In preferred embodiments, the cells of hematopoietic origin are B cells.

**[0061]** The present invention further encompasses methods and compositions for inhibiting or reducing proliferation of cells of hematopoietic origin, comprising, or alternatively consisting of, administering to an animal in which such inhibition or reduction is desired, a Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide in an amount effective to inhibit or reduce Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV-mediated cell proliferation. In preferred embodiments, the cells of hematopoietic origin are B cells.

**[0062]** The present invention further encompasses methods and compositions for decreasing activation of cells of hematopoietic origin, comprising, or alternatively consisting of, contacting an effective amount of Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide with cells of hematopoietic origin, wherein the effective amount of Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide decreases Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV-mediated activation of cells of hematopoietic origin. In preferred embodiments the cells of hematopoietic origin are B cells.

**[0063]** The present invention further encompasses methods and compositions for decreasing activation of cells of hematopoietic origin, comprising, or alternatively consisting of, administering to an animal in which such increase is desired, a Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide in an amount effective to decrease Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV-mediated activation of cells of hematopoietic origin. In preferred embodiments the cells of hematopoietic origin are B cells.

**[0064]** The present invention further encompasses methods and compositions for decreasing lifespan of B cells, comprising, or alternatively consisting of, contacting an effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV binding polypeptide decreases Neutrokin-alpha and/or Neutrokin-alphaSV-regulated lifespan of cells of hematopoietic origin. In preferred embodiments the cells of hematopoietic origin are B cells.

**[0065]** The present invention further encompasses methods and compositions for decreasing lifespan of cells of hematopoietic origin, comprising, or alternatively consisting of, administering to an animal in which such reduction is desired, a Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide in an amount effective to decrease Neutrokin-alpha and/or Neutrokin-alphaSV-regulated lifespan of cells of hematopoietic origin. In preferred embodiments the cells of hematopoietic origin are B cells.

**[0066]** The present invention also provides a screening method for identifying compounds capable of enhancing or inhibiting a cellular response induced by Neutrokin-alpha and/or Neutrokin-alphaSV which involves contacting cells which express Neutrokin-alpha and/or Neutrokin-alphaSV with the candidate compound, assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made in absence of the candidate compound; whereby, an increased cellular response over the standard indicates that the compound is an agonist and a decreased cellular response over the standard indicates that the compound is an antagonist.

**[0067]** In another embodiment, a method for identifying Neutrokin-alpha and/or Neutrokin-alphaSV receptors is provided, as well as a screening assay for agonists and antagonists using such receptors. This assay involves determining the effect a candidate compound has on Neutrokin-alpha and/or Neutrokin-alphaSV binding to the Neutrokin-alpha and/or Neutrokin-alphaSV receptor. In particular, the method involves contacting a Neutrokin-alpha and/or Neutrokin-alphaSV receptor with a Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide of the invention and a candidate compound and determining whether Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide binding to the Neutrokin-alpha and/or Neutrokin-alphaSV receptor is increased or decreased due to the presence of the candidate compound. The antagonists

may be employed to prevent septic shock, inflammation, cerebral malaria, activation of the HIV virus, graft-host rejection, bone resorption, rheumatoid arthritis, cachexia (wasting or malnutrition), immune system function, lymphoma, and autoimmune disorders (e.g., rheumatoid arthritis and systemic lupus erythematosus).

**[0068]** The present inventors have discovered that Neutrokin- $\alpha$  is expressed not only in cells of monocytic lineage, but also in kidney, lung, peripheral leukocyte, bone marrow, T cell lymphoma, B cell lymphoma, activated T cells, stomach cancer, smooth muscle, macrophages, and cord blood tissue. The present inventors have further discovered that Neutrokin- $\alpha$ SV appears to be expressed highly only in primary dendritic cells. For a number of disorders of these tissues and cells, such as tumor and tumor metastasis, infection of bacteria, viruses and other parasites, immunodeficiencies (e.g., chronic variable immunodeficiency), septic shock, inflammation, cerebral malaria, activation of the HIV virus, graft-host rejection, bone resorption, rheumatoid arthritis, autoimmune diseases (e.g., rheumatoid arthritis and systemic lupus erythematosus) and cachexia (wasting or malnutrition). It is believed that significantly higher or lower levels of Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV gene expression can be detected in certain tissues (e.g., bone marrow) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV gene expression level, i.e., the Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV expression level in tissue or bodily fluids from an individual not having the disorder. Thus, the invention provides a diagnostic method useful during diagnosis of a disorder, which involves: (a) assaying Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV gene expression level in cells or body fluid of an individual; (b) comparing the Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV gene expression level with a standard Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV gene expression level, whereby an increase or decrease in the assayed Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV gene expression level compared to the standard expression level is indicative of a disorder.

**[0069]** An additional embodiment of the invention is related to a method for treating an individual in need of an increased or constitutive level of Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an isolated

Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide of the invention or an agonist thereof.

[0070] A still further embodiment of the invention is related to a method for treating an individual in need of a decreased level of Neutrokin-alpha and/or Neutrokin-alphaSV activity in the body comprising, administering to such an individual a composition comprising a therapeutically effective amount of an Neutrokin-alpha and/or Neutrokin-alphaSV antagonist. Preferred antagonists for use in the present invention are Neutrokin-alpha-specific and/or Neutrokin-alphaSV-specific antibodies.

### ***Brief Description of the Figures***

[0071] The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

[0072] **Figures 1A and 1B** show the nucleotide (SEQ ID NO:1) and deduced amino acid (SEQ ID NO:2) sequences of Neutrokin-alpha. Amino acids 1 to 46 represent the predicted intracellular domain, amino acids 47 to 72 the predicted transmembrane domain (the double-underlined sequence), and amino acids 73 to 285, the predicted extracellular domain (the remaining sequence). Potential asparagine-linked glycosylation sites are marked in Figures 1A and 1B with a bolded asparagine symbol (N) in the Neutrokin-alpha amino acid sequence and a bolded pound sign (#) above the first nucleotide encoding that asparagine residue in the Neutrokin-alpha nucleotide sequence. Potential N-linked glycosylation sequences are found at the following locations in the Neutrokin-alpha amino acid sequence: N-124 through Q-127 (N-124, S-125, S-126, Q-127) and N-242 through C-245 (N-242, N-243, S-244, C-245).

[0073] Regions of high identity between Neutrokin-alpha, Neutrokin-alphaSV, TNF-alpha, TNF-beta, LT-beta, and the closely related Fas Ligand (an alignment of these sequences is presented in Figures 2A, 2B, 2C, and 2D) are underlined in Figures 1A and 1B. These regions are not limiting and are labeled as Conserved Domain (CD)-I, CD-II, CD-III, CD-IV, CD-V, CD-VI, CD-VII, CD-VIII, CD-IX, CD-X, and CD-XI in Figures 1A and 1B.

[0074] **Figures 2A, 2B, 2C, and 2D** show the regions of identity between the amino acid sequences of Neutrokin-alpha (SEQ ID NO:2) and Neutrokin-alphaSV (SEQ ID NO:19), and TNF-alpha ("TNFalpha" in Figures 2A, 2B, 2C, and 2D; GenBank No.

Z15026; SEQ ID NO:3), TNF-beta ("TNFbeta" in Figures 2A, 2B, 2C, and 2D; GenBank No. Z15026; SEQ ID NO:4), Lymphotoxin-beta ("LTbeta" in Figures 2A, 2B, 2C, and 2D; GenBank No. L11016; SEQ ID NO:5), and FAS ligand ("FASL" in Figures 2A, 2B, 2C, and 2D; GenBank No. U11821; SEQ ID NO:6), determined by the "MegAlign" routine which is part of the computer program called "DNA\*STAR." Residues that match the consensus are shaded.

**[0075]** Figure 3 shows an analysis of the Neutrokin-alpha amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown, as predicted for the amino acid sequence of SEQ ID NO:2 using the default parameters of the recited computer programs. In the "Antigenic Index - Jameson-Wolf" graph, the indicate location of the highly antigenic regions of Neutrokin-alpha i.e., regions from which epitope-bearing peptides of the invention may be obtained. Antigenic polypeptides include from about Phe-115 to about Leu-147, from about Ile-150 to about Tyr-163, from about Ser-171 to about Phe-194, from about Glu-223 to about Tyr-246, and from about Ser-271 to about Phe-278, of the amino acid sequence of SEQ ID NO:2.

**[0076]** The data presented in Figure 3 are also represented in tabular form in Table I. The columns are labeled with the headings "Res", "Position", and Roman Numerals I-XIV. The column headings refer to the following features of the amino acid sequence presented in Figure 3, and Table I: "Res": amino acid residue of SEQ ID NO:2 and Figures 1A and 1B; "Position": position of the corresponding residue within SEQ ID NO:2 and Figures 1A and 1B; I: Alpha, Regions - Garnier-Robson; II: Alpha, Regions - Chou-Fasman; III: Beta, Regions - Garnier-Robson; IV: Beta, Regions - Chou-Fasman; V: Turn, Regions - Garnier-Robson; VI: Turn, Regions - Chou-Fasman; VII: Coil, Regions - Garnier-Robson; VIII: Hydrophilicity Plot - Kyte-Doolittle; IX: Hydrophobicity Plot - Hopp-Woods; X: Alpha, Amphipathic Regions - Eisenberg; XI: Beta, Amphipathic Regions - Eisenberg; XII: Flexible Regions - Karplus-Schulz; XIII: Antigenic Index - Jameson-Wolf; and XIV: Surface Probability Plot - Emini.

**[0077]** Figures 4A, 4B, and 4C show the alignment of the Neutrokin-alpha nucleotide sequence determined from the human cDNA deposited in ATCC No. 97768 with related human cDNA clones of the invention which have been designated HSOAD55 (SEQ ID NO:7), HSLAH84 (SEQ ID NO:8) and HLTBM08 (SEQ ID NO:9).

[0078] **Figures 5A and 5B** shows the nucleotide (SEQ ID NO:18) and deduced amino acid (SEQ ID NO:19) sequences of the Neutrokin- $\alpha$ SV protein. Amino acids 1 to 46 represent the predicted intracellular domain, amino acids 47 to 72 the predicted transmembrane domain (the double-underlined sequence), and amino acids 73 to 266, the predicted extracellular domain (the remaining sequence). Potential asparagine-linked glycosylation sites are marked in Figures 5A and 5B with a bolded asparagine symbol (N) in the Neutrokin- $\alpha$ SV amino acid sequence and a bolded pound sign (#) above the first nucleotide encoding that asparagine residue in the Neutrokin- $\alpha$ SV nucleotide sequence. Potential N-linked glycosylation sequences are found at the following locations in the Neutrokin- $\alpha$ SV amino acid sequence: N-124 through Q-127 (N-124, S-125, S-126, Q-127) and N-223 through C-226 (N-223, N-224, S-225, C-226). Antigenic polypeptides include from about Pro-32 to about Leu-47, from about Glu-116 to about Ser-143, from about Phe-153 to about Tyr-173, from about Pro-218 to about Tyr-227, from about Ala-232 to about Gln-241; from about Ile-244 to about Ala-249; and from about Ser-252 to about Val-257 of the amino acid sequence of SEQ ID NO:19.

[0079] Regions of high identity between Neutrokin- $\alpha$ , Neutrokin- $\alpha$ SV, TNF- $\alpha$ , TNF- $\beta$ , LT- $\beta$ , and the closely related Fas Ligand (an alignment of these sequences is presented in Figure 2) are underlined in Figures 1A and 1B. These conserved regions (of Neutrokin- $\alpha$  and Neutrokin- $\alpha$ SV) are labeled as Conserved Domain (CD)-I, CD-II, CD-III, CD-V, CD-VI, CD-VII, CD-VIII, CD-IX, CD-X, and CD-XI in Figures 5A and 5B. Neutrokin- $\alpha$ SV does not contain the sequence of CD-IV described in the legend of Figures 1A and 1B.

[0080] An additional alignment of the Neutrokin- $\alpha$  polypeptide sequence (SEQ ID NO:2) with APRIL, TNF  $\alpha$ , and LT  $\alpha$  is presented in Figures 7A-1 and 7A-2. In Figures 7A-1 and 7A-2, beta sheet regions are indicated as described below in the legend to Figures 7A-1 and 7A-2.

[0081] **Figure 6** shows an analysis of the Neutrokin- $\alpha$ SV amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown, as predicted for the amino acid sequence of SEQ ID NO:19 using the default parameters of the recited computer programs. The location of the highly antigenic regions of the Neutrokin- $\alpha$  protein, i.e., regions from which epitope-bearing peptides of the invention may be



obtained is indicated in the "Antigenic Index - Jameson-Wolf" graph. Antigenic polypeptides include, but are not limited to, a polypeptide comprising amino acid residues from about Pro-32 to about Leu-47, from about Glu-116 to about Ser-143, from about Phe-153 to about Tyr-173, from about Pro-218 to about Tyr-227, from about Ser-252 to about Thr-258, from about Ala-232 to about Gln-241; from about Ile-244 to about Ala-249; and from about Ser-252 to about Val-257, of the amino acid sequence of SEQ ID NO:19.

**[0082]** The data shown in Figure 6 can be easily represented in tabular format similar to the data shown in Table I. Such a tabular representation of the exact data disclosed in Figure 6 can be generated using the MegAlign component of the DNA\*STAR computer sequence analysis package set on default parameters. This is the identical program that was used to generate Figures 3 and 6 of the present application.

**[0083] Figures 7A-1 and 7A-2.** The amino-acid sequence of Neutrokin- $\alpha$  and alignment of its predicted ligand-binding domain with those of APRIL, TNF- $\alpha$ , and LT- $\alpha$  (specifically, amino acid residues 115-250 of the human APRIL polypeptide (SEQ ID NO:20; GenBank Accession No. AF046888 (nucleotide) and AAC6132 (protein)), amino acid residues 88-233 of TNF  $\alpha$  (SEQ ID NO:3; GenBank Accession No. Z15026), and LT  $\alpha$  ((also designated TNF- $\beta$ ) amino acid residues 62-205 of SEQ ID NO:4; GenBank Accession No. Z15026)). The predicted membrane-spanning region of Neutrokin- $\alpha$  is indicated and the site of cleavage of Neutrokin- $\alpha$  is depicted with an arrow. Sequences overlaid with lines (A thru H) represent predicted beta-pleated sheet regions.

**[0084] Figure 7B.** Expression of Neutrokin- $\alpha$  mRNA. Northern hybridization analysis was performed using the Neutrokin- $\alpha$  orf as a probe on blots of poly (A)+ RNA (Clontech) from a spectrum of human tissue types and a selection of cancer cell lines. A 2.6 kb Neutrokin- $\alpha$  mRNA was detected at high levels in placenta, heart, lung, fetal liver, thymus, and pancreas. The 2.6 kb Neutrokin- $\alpha$  mRNA was also detected in HL-60 and K562 cell lines.

**[0085] Figures 8A, 8B and 8C.** Neutrokin- $\alpha$  expression increases following activation of human monocytes by IFN- $\gamma$ . **Figures 8A and 8B.** Flow cytometric analysis of Neutrokin- $\alpha$  protein expression on *in vitro* cultured monocytes. Purified monocytes were cultured for 3 days in presence or absence of IFN- $\gamma$  (100 U/ml).

Cells were then stained with a Neutrokin- $\alpha$ -specific mAb (2E5) (solid lines) or an isotype-matched control (IgG1) (dashed lines). Comparable results were obtained with monocytes purified from three different donors in three independent experiments. **Figure 8C.** Neutrokin- $\alpha$ -specific TaqMan primers were prepared and used to assess the relative Neutrokin- $\alpha$  mRNA expression levels in unstimulated and IFN- $\gamma$  (100 U/mL) treated monocytes. Nucleotide sequences of the TaqMan primers are as follows: (a) Probe: 5'-CCA CCA GCT CCA GGA GAA GGC AAC TC-3' (SEQ ID NO:24); (b) 5' amplification primer: 5'-ACC GCG GGA CTG AAA ATC T-3' (SEQ ID NO:25); and (c) 3' amplification primer: 5'-CAC GCT TAT TTC TGC TGT TCT GA-3' (SEQ ID NO:26).

[0086] **Figures 9A and 9B.** Neutrokin- $\alpha$  is a potent B lymphocyte stimulator. **Figure 9A.** The biological activity of Neutrokin- $\alpha$  was assessed in a standard B-lymphocyte co-stimulation assay utilizing *Staphylococcus aureus* cowan 1 SAC as the priming agent. SAC alone yielded background counts of 1427  $\pm$  316. Values are reported as mean  $\pm$  standard deviation of triplicate wells. Similar results were obtained using recombinant Neutrokin- $\alpha$  purified from stable CHO transfectants and transiently transfected HEK 293T cells. **Figure 9B.** Proliferation of tonsillar B cells with Neutrokin- $\alpha$  and co-stimulation with anti-IgM. The bioassay was performed as described for SAC with the exception that individual wells were pre-coated with goat anti-human IgM antibody at 10 micrograms/mL in PBS.

[0087] **Figures 10A, 10B, 10C, 10D, 10E, 10F and 10G.** Neutrokin- $\alpha$  receptor expression among normal human peripheral blood mononuclear cells and tumor cell lines. **Figures 10A, 10B, 10C, 10D and 10E.** Human peripheral blood nucleated cells were obtained from normal volunteers and isolated by density gradient centrifugation. Cells were stained with biotinylated Neutrokin- $\alpha$  followed by PE-conjugated streptavidin and FITC or PerCP coupled mAbs specific for CD3, CD20, CD14, CD56, and CD66b. Cells were analyzed on a Becton Dickinson FACScan using the CellQuest software. Data represent one of four independent experiments. **Figures 10F and 10G.** Neutrokin- $\alpha$  binding to histiocytic cell line U-937 and the myeloma line IM-9.

[0088] **Figures 11A, 11B, 11C, 11D, 11E, and 11F.** *In vivo* effects of Neutrokin- $\alpha$  administration in BALB/cAnNCR mice. **Figure 11A.** Formalin-fixed spleens were paraffin embedded and 5 micrometer sections stained with hematoxylin and eosin (upper panels). The lower panels are sections taken from the same animals stained with anti-

CD45R(B220) mAb and developed with horseradish-peroxidase coupled rabbit anti-rat Ig (mouse adsorbed) and the substrate diaminobenzidine tetrahydrochloride (DAB). Slides were counter-stained with Mayer's hematoxylin. CD45R(B220) expressing cells appear brown. **Figures 11B and 11C.** Flow cytometric analyses of normal (left panel) and Neutrokinine-alpha-treated (right panel) stained with PE-CD45R(B220) and FITC-ThB (Ly6D). **Figures 11D, 11E, and 11F.** Serum IgM, IgG, and IgA levels in normal and Neutrokinine-alpha treated mice.

**[0089] Figure 12.** Effect of  $^{131}\text{I}$ -labeled Neutrokinine-alpha (lot TX1) on the survival of BCL1 tumor-bearing BALB/c mice. Survival curve expressed in terms of survival probability vs. time. Day 0 is the first day of tumor cell injection. Differences among the treatment groups were analyzed using the Log Rank Test for equality. Treatment with  $^{131}\text{I}$ -labeled Neutrokinine-alpha (LR131 in figure) at doses of either 11.9 or 15.3 mCi/kg (red and blue dotted lines, respectively) significantly prolonged survival ( $p = 0.0162$  and  $p = 0.0052$ , respectively) compared with vehicle-treated controls (black solid line). In the group of mice that did not have BCL1 tumors but did receive 15.3 mCi/kg of  $^{131}\text{I}$ -labeled Neutrokinine-alpha, 10% of the mice died (yellow dashed line).

**[0090] Figure 13.** Effect of  $^{131}\text{I}$ -labeled Neutrokinine-alpha (lot TX2) on the survival of BCL1 tumor-bearing BALB/c mice. Survival curve expressed in terms of survival probability vs. time. Day 0 is the first day of tumor cell injection. Differences among the treatment groups were analyzed using the Log Rank Test for equality. Treatment with  $^{131}\text{I}$ -labeled Neutrokinine-alpha (LR131 in figure) at a dose of 17.5 mCi/kg (dashed line) significantly prolonged survival ( $p = 0.0348$ ) compared with the vehicle-treated controls (black solid line). In the group of mice that did not have BCL1 tumors but did receive  $^{131}\text{I}$ -labeled Neutrokinine-alpha, 12.5% of the mice died (dotted line).

**[0091] Figure 14.** Effect of  $^{131}\text{I}$ -labeled Neutrokinine-alpha (lot TX3) on the survival of BCL1 tumor-bearing BALB/c mice. Survival curve expressed in terms of survival probability vs. time. Day 0 is the day the tumor cells were injected. Differences among the treatment groups were analyzed using the Log Rank Test for equality. Treatment with  $^{131}\text{I}$ -labeled Neutrokinine-alpha (LR131 in figure) at a dose of 37.7 mCi/kg (dashed line) significantly prolonged survival ( $p = 0.0212$ ) compared to the vehicle-treated controls (solid line). In the group of mice that did not have BCL1 tumors but did receive  $^{131}\text{I}$ -labeled Neutrokinine-alpha, 12.5% of the mice died (dotted line).

[0092] Figure 15 shows a plasmid map of the pML124 vector. The sequence of this vector is shown in SEQ ID NO:52.

[0093] Figure 16 shows a plasmid map of the pML124 vector containing the MBPss-Neutrokin- $\alpha$  fusion. The sequence of this vector is shown in SEQ ID NO:53.

### ***Detailed Description***

[0094] The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding a Neutrokin- $\alpha$  polypeptides having the amino acid sequences shown in Figures 1A and 1B (SEQ ID NO:2), which was determined by sequencing a cDNA clone. The nucleotide sequence shown in Figures 1A and 1B (SEQ ID NO:1) was obtained by sequencing the HNEDU15 clone, which was deposited on October 22, 1996 at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, and assigned ATCC Accession No. 97768. The deposited clone is contained in the pBluescript SK(-) plasmid (Stratagene, La Jolla, CA). The ATCC deposits were made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedure.

[0095] The present invention also provides isolated nucleic acid molecules comprising a polynucleotide encoding Neutrokin- $\alpha$ SV polypeptides having the amino acid sequences shown in Figures 5A and 5B (SEQ ID NO:19), which was determined by sequencing a cDNA clone. The nucleotide sequence shown in Figures 5A and 5B (SEQ ID NO:18) was obtained by sequencing the HDPMC52 clone, which was deposited on December 10, 1998 at the American Type Culture Collection, and assigned ATCC Accession No. 203518. The deposited clone is contained in the pBluescript SK(-) plasmid (Stratagene, La Jolla, CA). The ATCC deposits were made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedure.

[0096] The Neutrokin- $\alpha$  and Neutrokin- $\alpha$  polypeptides of the present invention share sequence homology with the translation products of the human mRNAs for TNF- $\alpha$ , TNF- $\beta$ , LT $\beta$ , Fas ligand, APRIL, and LT $\alpha$ . (See, Figures 2A, 2B, 2C, 2D, 7A-1 and 7A-2). As noted above, TNF- $\alpha$  is thought to be an important

cytokine that plays a role in cytotoxicity, necrosis, apoptosis, costimulation, proliferation, lymph node formation, immunoglobulin class switch, differentiation, antiviral activity, and regulation of adhesion molecules and other cytokines and growth factors.

#### Nucleic Acid Molecules

**[0097]** Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined using an automated DNA sequencer (such as the Model 373 from Applied Biosystems, Inc., Foster City, CA), and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by this automated approach, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by automation are typically at least about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. The actual sequence can be more precisely determined by other approaches including manual DNA sequencing methods well known in the art. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

**[0098]** By "nucleotide sequence" of a nucleic acid molecule or polynucleotide is intended, for a DNA molecule or polynucleotide, a sequence of deoxyribonucleotides, and for an RNA molecule or polynucleotide, the corresponding sequence of ribonucleotides (A, G, C and U), where each thymidine deoxyribonucleotide (T) in the specified deoxyribonucleotide sequence is replaced by the ribonucleotide uridine (U).

**[0099]** Using the information provided herein, such as the nucleotide sequence in Figures 1A and 1B, a nucleic acid molecule of the present invention encoding a Neutrokin- $\alpha$  polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention, the nucleic acid molecule described in Figures 1A and 1B (SEQ ID NO:1) was discovered in a cDNA library derived from neutrophils. Expressed

sequence tags corresponding to a portion of the Neutrokin-alpha cDNA were also found in kidney, lung, peripheral leukocyte, bone marrow, T cell lymphoma, B cell lymphoma, activated T cells, stomach cancer, smooth muscle, macrophages, and cord blood tissue. In addition, using the nucleotide information provided in Figures 5A and 5B, a nucleic acid molecule of the present invention encoding a Neutrokin-alphaSV polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention, the nucleic acid molecule described in Figures 5A and 5B (SEQ ID NO:18) was discovered in a cDNA library derived from primary dendritic cells.

**[0100]** The Neutrokin-alpha plasmid HNEDU15 deposited as ATCC Accession No. 97768 contains an open reading frame encoding a protein of about 285 amino acid residues, a predicted intracellular domain of about 46 amino acids (amino acid residues from about 1 to about 46 in Figures 1A and 1B (SEQ ID NO:2)), a predicted transmembrane domain of about 26 amino acids (underlined amino acid residues from about 47 to about 72 in Figures 1A and 1B (SEQ ID NO:2)), a predicted extracellular domain of about 213 amino acids (amino acid residues from about 73 to about 285 in Figures 1A and 1B (SEQ ID NO:2)); and a deduced molecular weight of about 31 kDa. The Neutrokin-alpha polypeptide shown in Figures 1A and 1B (SEQ ID NO:2) is about 20% similar and about 10 % identical to human TNF-alpha, which can be accessed on GenBank as Accession No. 339764.

**[0101]** The Neutrokin-alphaSV plasmid HDPMC52, deposited as ATCC Accession No. 203518, contains a predicted open reading frame encoding a protein of about 266 amino acid residues, a predicted intracellular domain of about 46 amino acids (amino acid residues from about 1 to about 46 in Figures 5A and 5B (SEQ ID NO:19)), a predicted transmembrane domain of about 26 amino acids (underlined amino acid residues from about 47 to about 72 in Figures 5A and 5B (SEQ ID NO:19)), a predicted extracellular domain of about 194 amino acids (amino acid residues from about 73 to about 266 in Figures 5A and 5B (SEQ ID NO:19)); and a deduced molecular weight of about 29 kDa. The Neutrokin-alphaSV polypeptide shown in Figures 5A and 5B (SEQ ID NO:19) is about 33.9% similar and about 22.0% identical to human TNF-alpha which can be accessed on GenBank as Accession No. 339764. As one of ordinary skill would appreciate, due to the possibilities of sequencing errors discussed above, the actual

complete Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides encoded by the deposited cDNAs, which comprise about 285 and 266 amino acids, respectively, may be somewhat shorter. In particular, the determined Neutrokin- $\alpha$  and Neutrokin- $\alpha$ SV coding sequences contain a common second methionine codon which may serve as an alternative start codon for translation of the open reading frame, at nucleotide positions 210-212 in Figures 1A and 1B (SEQ ID NO:1) and at nucleotide positions 64-66 in Figures 5A and 5B (SEQ ID NO:18). More generally, the actual open reading frame may be anywhere in the range of  $\pm 20$  amino acids, more likely in the range of  $\pm 10$  amino acids, of that predicted from either the first or second methionine codon from the N-terminus shown in Figures 1A and 1B (SEQ ID NO:1) and in Figures 5A and 5B (SEQ ID NO:18). It will further be appreciated that, the polypeptide domains described herein have been predicted by computer analysis, and accordingly, that depending on the analytical criteria used for identifying various functional domains, the exact "address" of the extracellular, intracellular and transmembrane domains of the Neutrokin- $\alpha$  and Neutrokin- $\alpha$ SV polypeptides may differ slightly. For example, the exact location of the Neutrokin- $\alpha$  and Neutrokin- $\alpha$ SV extracellular domains in Figures 1A and 1B (SEQ ID NO:2) and Figures 5A and 5B (SEQ ID NO:19) may vary slightly (e.g., the address may "shift" by about 1 to about 20 residues, more likely about 1 to about 5 residues) depending on the criteria used to define the domain. In this case, the ends of the transmembrane domains and the beginning of the extracellular domains were predicted on the basis of the identification of the hydrophobic amino acid sequence in the above indicated positions, as shown in Figures 3 and 6 and in Table I. In any event, as discussed further below, the invention further provides polypeptides having various residues deleted from the N-terminus and/or C-terminus of the complete polypeptides, including polypeptides lacking one or more amino acids from the N-termini of the extracellular domains described herein, which constitute soluble forms of the extracellular domains of the Neutrokin- $\alpha$  and Neutrokin- $\alpha$ SV polypeptides.

[0102] As indicated, nucleic acid molecules and polynucleotides of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA or RNA may

be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

[0103] By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule (DNA or RNA), which has been removed from its native environment. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the DNA molecules of the present invention. However, a nucleic acid contained in a clone that is a member of a library (e.g., a genomic or cDNA library) that has not been isolated from other members of the library (e.g., in the form of a homogeneous solution containing the clone and other members of the library) or a chromosome isolated or removed from a cell or a cell lysate (e.g., a "chromosome spread", as in a karyotype), is not "isolated" for the purposes of this invention. As discussed further herein, isolated nucleic acid molecules according to the present invention may be produced naturally, recombinantly, or synthetically.

[0104] Isolated nucleic acid molecules of the present invention include DNA molecules comprising, or alternatively consisting of, an open reading frame (ORF) with an initiation codon at positions 147-149 of the nucleotide sequence shown in Figures 1A and 1B (SEQ ID NO:1). In addition, isolated nucleic acid molecules of the invention include DNA molecules which comprise, or alternatively consist of, a sequence substantially different from those described above, but which due to the degeneracy of the genetic code, still encodes the Neutrokin- $\alpha$  protein. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate the degenerate variants described above. In another embodiment, the invention provides isolated nucleic acid molecules comprising, or alternatively consisting of, a sequence encoding the Neutrokin- $\alpha$  polypeptide having an amino acid sequence encoded by the cDNA contained in the plasmid having ATCC accession number 97768. Preferably, this nucleic acid molecule comprises, or alternatively consists of a sequence encoding the extracellular domain the mature or soluble polypeptide sequence of the polypeptide encoded by the cDNA contained in the plasmid having ATCC accession number 97768.



**[0105]** Isolated nucleic acid molecules of the present invention also include DNA molecules comprising an open reading frame (ORF) with an initiation codon at positions 1-3 of the nucleotide sequence shown in Figures 5A and 5B (SEQ ID NO:18). In addition, isolated nucleic acid molecules of the invention include DNA molecules which comprise, or alternatively consist of, a sequence substantially different from those described above, but which due to the degeneracy of the genetic code, still encodes the Neutrokin-alphaSV polypeptide. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate the degenerate variants described above. In another embodiment, the invention provides isolated nucleic acid molecules comprising, or alternatively consisting of, a sequence encoding the Neutrokin-alphaSV polypeptide having an amino acid encoded by the cDNA contained in the plasmid having ATCC accession number 203518. Preferably, this nucleic acid molecule comprises, or alternatively consists of, a sequence encoding the extracellular domain or the mature soluble polypeptide sequence of the polypeptide encoded by the cDNA contained in the plasmid having ATCC accession number 203518.

**[0106]** The invention further provides an isolated nucleic acid molecule comprising, or alternatively consisting of, the nucleotide sequence shown in Figures 1A and 1B (SEQ ID NO:1) or the nucleotide sequence of the Neutrokin-alpha cDNA contained in the plasmid having ATCC accession number 97768, or a nucleic acid molecule having a sequence complementary to one of the above sequences. In addition, the invention provides an isolated nucleic acid molecule comprising, or alternatively, consisting of, the nucleotide sequence shown in Figures 5A and 5B (SEQ ID NO:18) or the nucleotide sequence of the Neutrokin-alpha SV cDNA contained in the plasmid having ATCC accession number 203518, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, have uses which include, but are not limited to, as probes for gene mapping by *in situ* hybridization with chromosomes, and for detecting expression of the Neutrokin-alpha and Neutrokin-alphaSV in human tissue, for instance, by Northern or Western blot analysis.

**[0107]** In one embodiment, the polynucleotides of the invention comprise, or alternatively consist of, the sequence shown in SEQ ID NO:22. The sequence provided as SEQ ID NO:22 was constructed from several overlapping mouse EST sequences obtained from GenBank (AI182472, AA422749, AA254047, and AI122485). The EST sequences

were aligned to generate the Neutrokin- $\alpha$ -like polynucleotide sequence provided as SEQ ID NO:22. The amino acid sequence resulting from the translation of SEQ ID NO:22 is provided as SEQ ID NO:23. Fragments, variants, and derivatives of the sequences provided as SEQ ID NO:22 and SEQ ID NO:23 are also encompassed by the invention.

[0108] In another embodiment, the polynucleotides of the invention comprise, or alternatively consist of, the sequence shown in SEQ ID NO:27, and/or a sequence encoding the amino acid sequence disclosed in SEQ ID NO:28, fragments, variants, and derivatives thereof. These polynucleotides are also encompassed by the invention. For example, certain embodiments of the invention are directed to polynucleotides comprising, or alternatively consisting of, a sequence encoding a polypeptide sequence that is at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to amino acids 68-219 of SEQ ID NO:28. The amino acid sequence resulting from the translation of SEQ ID NO:27 is provided as SEQ ID NO:28. Polypeptides comprising, or alternatively consisting of, the amino acid sequence of SEQ ID NO:28, and fragments, variants, and derivatives of the sequence provided as SEQ ID NO:28 are also encompassed by the invention. For example, certain embodiments of the invention are directed to polypeptides comprising, or alternatively consisting of, a polypeptide sequence that is at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to amino acids 68-219 of SEQ ID NO:28. A nucleic acid molecule having the sequence provided as SEQ ID NO:27 was obtained by RT-PCR from cyanomolgous monkey (i.e., *Macaca irus*) PBMC using two degenerate primers. Briefly, total RNA was prepared from cyanomolgous monkey PBMC by using Trizol (available from Life Technologies, Inc., Rockville, MD) according to the manufacturer's protocol. Then a single stranded cDNA was synthesized from the cyanomolgous monkey PBMC preparation using standard methods with an oligo-dT primer. Neutrokin- $\alpha$ -specific primers were designed based on the conserved region between the mouse and human Neutrokin- $\alpha$  molecules (SEQ ID NOs:22 and 1, respectively). A cyanomolgous monkey Neutrokin- $\alpha$  nucleic acid molecule was then generated by PCR using the cDNA template in combination with the following two degenerate oligonucleotide primers. 5' primer: 5'-TAC CAG ITG GCI GCC ITG CAA G-3' (SEQ ID NO:35) and 3' primer: 5'-GTI ACA GCA GTT TIA IIG

CAC C-3' (SEQ ID NO:36). In the sequence of the degenerate primers (SEQ ID NOs:35 and 36), "I" represents deoxyinosine or dideoxyinosine.

**[0109]** In another embodiment, the polynucleotides of the invention comprise, or alternatively consist of, the sequence shown in SEQ ID NO:29, and/or a sequence encoding the amino acid sequence disclosed in SEQ ID NO:30, fragments, variants, and derivatives thereof. These polynucleotides are also encompassed by the invention. For example, certain embodiments of the invention are directed to polynucleotides comprising, or alternatively consisting of, a sequence encoding a polypeptide sequence that is at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to amino acids 68-219 of SEQ ID NO:30. The amino acid sequence resulting from the translation of SEQ ID NO:29 is provided as SEQ ID NO:30. Polypeptides comprising, or alternatively consisting of, the amino acid sequence of SEQ ID NO:30, and fragments, variants, and derivatives of the sequences provided as SEQ ID NO:29 and SEQ ID NO:30 are also encompassed by the invention. For example, certain embodiments of the invention are directed to polypeptides comprising, or alternatively consisting of, a polypeptide sequence that is at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to amino acids 68-219 of SEQ ID NO:30. A nucleic acid molecule having the sequence provided as SEQ ID NO:29 was obtained by RT-PCR from rhesus monkey PBMC using two degenerate primers. Briefly, total RNA was prepared from rhesus monkey PBMC by using Trizol (available from Life Technologies, Inc., Rockville, MD) according to the manufacturer's protocol. Then a single stranded cDNA was synthesized from the rhesus monkey PBMC preparation using standard methods with an oligo-dT primer. Neutrokin- $\alpha$ -specific primers were designed based on the conserved region between the mouse and human Neutrokin- $\alpha$  molecules (SEQ ID NOs:22 and 1, respectively). A rhesus monkey Neutrokin- $\alpha$  nucleic acid molecule was then generated by PCR using the cDNA template in combination with the following two degenerate oligonucleotide primers. 5' primer: 5'-TAC CAG ITG GCI GCC ITG CAA G-3' (SEQ ID NO:35) and 3' primer: 5'-GTI ACA GCA GTT TIA IIG CAC C-3' (SEQ ID NO:36). In the sequence of the degenerate primers (SEQ ID NOs:35 and 36), "I" represents deoxyinosine or dideoxyinosine.

**[0110]** The invention also provides nucleic acid molecules having nucleotide sequences related to extensive portions of SEQ ID NO:1 and SEQ ID NO:18 which have

been determined from the following related cDNA clones: HSOAD55 (SEQ ID NO:7), HSLAH84 (SEQ ID NO:8), and HLTBM08 (SEQ ID NO:9).

[0111] The present invention is further directed to nucleic acid molecules encoding portions of the nucleotide sequences described herein, as well as to fragments of the isolated nucleic acid molecules described herein. In one embodiment, the invention provides a polynucleotide having a nucleotide sequence representing the portion of SEQ ID NO:1 which consists of the nucleotides at positions 1-1001 of SEQ ID NO:1. In another embodiment, the invention provides a polynucleotide having a nucleotide sequence representing the portion of SEQ ID NO:18 which consists of positions 1-798 of SEQ ID NO:18.

[0112] The present invention is further directed to fragments of the nucleic acid molecules (i.e. polynucleotides) described herein. By a fragment of a nucleic acid molecule having, for example, the nucleotide sequence of the cDNA contained in the plasmid having ATCC accession number 97768, a nucleotide sequence encoding the polypeptide sequence encoded by the cDNA contained in the plasmid having ATCC accession number 97768, the nucleotide sequence of SEQ ID NO:1, a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:2, the nucleotide sequence of the cDNA contained in the plasmid having ATCC accession number 203518, a nucleotide sequence encoding the polypeptide sequence encoded by the cDNA contained in the plasmid having ATCC accession number 203518, the nucleotide sequence of SEQ ID NO:18, a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:19, or the complementary strand thereto, is intended fragments at least 15 nt, and more preferably at least 20 nt or at least 25 nt, still more preferably at least 30 nt, and even more preferably, at least 40, 50, 100, 150, 200, 250, 300, 325, 350, 375, 400, 450, or 500 nt in length. These fragments have numerous uses which include, but are not limited to, diagnostic probes and primers as discussed herein. Of course, larger fragments, such as those of 501-1500 nt in length are also useful according to the present invention as are fragments corresponding to most, if not all, of the nucleotide sequences of the cDNA contained in the plasmid having ATCC accession number 97768, the nucleotide sequence of SEQ ID NO:1, the nucleotide sequences of the cDNA contained in the plasmid having ATCC accession number 203518, and the nucleotide sequence of SEQ ID NO:18. Preferred nucleic acid fragments of the present invention include nucleic acid molecules

encoding polypeptides comprising, or alternatively, consisting of, epitope-bearing portions of the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide as identified in Figures 1A and 1B (SEQ ID NO:2) and in Figures 5A and 5B (SEQ ID NO:19), respectively, and described in more detail below. Polypeptides encoded by these polynucleotide fragments are also encompassed by the invention.

**[0113]** Also by a fragment of a nucleic acid molecule having, for example, the nucleotide sequence of SEQ ID NO:21, the nucleotide sequence of SEQ ID NO:22, the nucleotide sequence of SEQ ID NO:27, the nucleotide sequence of SEQ ID NO:29, the nucleotide sequence of SEQ ID NO:37, a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:23, a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:28, a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:30, a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:38, a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:39, a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:40, a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:41, a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:42, a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:43, a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:44, or the complementary strands thereof, is intended fragments at least 15 nt, and more preferably at least 20 nt or at least 25 nt, still more preferably at least 30 nt, and even more preferably, at least 40, 50, 100, 150, 200, 250, 300, 325, 350, 375, 400, 450, or 500 nt in length. These fragments have numerous uses which include, but are not limited to, diagnostic probes and primers as discussed herein. Of course, larger fragments, such as those of 501-1500 nt in length are also useful according to the present invention as are fragments corresponding to most, if not all, of the nucleotide sequence of SEQ ID NO:21, the nucleotide sequence of SEQ ID NO:22, the nucleotide sequence of SEQ ID NO:27, the nucleotide sequence of SEQ ID NO:29, the nucleotide sequence of SEQ ID NO:37, a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:23, a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:28, a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:30 a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:38, a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:39, a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:40, a nucleotide sequence encoding the polypeptide sequence of SEQ ID

NO:41, a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:42, a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:43, a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:44, or the complementary strands thereof. Polypeptides encoded by these polynucleotide fragments are also encompassed by the invention.

**[0114]** Representative examples of Neutrokin- $\alpha$  polynucleotide fragments of the invention include, for example, fragments that comprise, or alternatively, consist of, a sequence from about nucleotide 1 to 50, 51 to 100, 101 to 146, 147 to 200, 201 to 250, 251 to 300, 301 to 350, 351 to 400, 401 to 450, 451 to 500, 501 to 550, 551 to 600, 600 to 650, 651 to 700, 701 to 750, 751 to 800, 800 to 850, 851 to 900, 901 to 950, 951 to 1000, 1001 to 1050, and/or 1051 to 1082, of SEQ ID NO:1, or the complementary strand thereto, or the cDNA contained in the plasmid having ATCC accession number 97768. In this context "about" includes the particularly recited ranges, and ranges that are larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini.

**[0115]** Representative examples of Neutrokin- $\alpha$ SV polynucleotide fragments of the invention include, for example, fragments that comprise, or alternatively, consist of, a sequence from about nucleotide 1 to 50, 51 to 100, 101 to 150, 151 to 200, 201 to 250, 251 to 300, 301 to 350, 351 to 400, 401 to 450, 451 to 500, 501 to 550, 551 to 600, 600 to 650, 651 to 700, 701 to 750, 751 to 800, 800 to 850, and/or 851 to 900 of SEQ ID NO:18, or the complementary strand thereto, or the cDNA contained in the plasmid having ATCC accession number 203518. In this context "about" includes the particularly recited ranges, and ranges that are larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini.

**[0116]** In certain preferred embodiments, polynucleotide of the invention comprise, or alternatively, consist of, nucleotide residues 571-627, 580-627, 590-627, 600-627, 610-627, 571-620, 580-620, 590-620, 600-620, 571-610, 580-610, 590-610, 571-600, 580-600, and/or 571-590 of SEQ ID NO:1.

**[0117]** In certain other preferred embodiments, polynucleotides of the invention comprise, or alternatively, consist of nucleotide residues 1-879, 25-879, 50-879, 75-879, 100-879, 125-879, 150-879, 175-879, 200-879, 225-879, 250-879, 275-879, 300-879, 325-879, 350-879, 375-879, 400-879, 425-879, 450-879, 475-879, 500-879, 525-879, 550-879, 575-879, 600-879, 625-879, 650-879, 675-879, 700-879, 725-879, 750-879,

775-879, 800-879, 825-879, 850-879, 1-850, 25-850, 50-850, 75-850, 100-850, 125-850, 150-850, 175-850, 200-850, 225-850, 250-850, 275-850, 300-850, 325-850, 350-850, 375-850, 400-850, 425-850, 450-850, 475-850, 500-850, 525-850, 550-850, 575-850, 600-850, 625-850, 650-850, 675-850, 700-850, 725-850, 750-850, 775-850, 800-850, 825-850, 1-825, 25-825, 50-825, 75-825, 100-825, 125-825, 150-825, 175-825, 200-825, 225-825, 250-825, 275-825, 300-825, 325-825, 350-825, 375-825, 400-825, 425-825, 450-825, 475-825, 500-825, 525-825, 550-825, 575-825, 600-825, 625-825, 650-825, 675-825, 700-825, 725-825, 750-825, 775-825, 800-825, 1-800, 25-800, 50-800, 75-800, 100-800, 125-800, 150-800, 175-800, 200-800, 225-800, 250-800, 275-800, 300-800, 325-800, 350-800, 375-800, 400-800, 425-800, 450-800, 475-800, 500-800, 525-800, 550-800, 575-800, 600-800, 625-800, 650-800, 675-800, 700-800, 725-800, 750-800, 775-800, 1-775, 25-775, 50-775, 75-775, 100-775, 125-775, 150-775, 175-775, 200-775, 225-775, 250-775, 275-775, 300-775, 325-775, 350-775, 375-775, 400-775, 425-775, 450-775, 475-775, 500-775, 525-775, 550-775, 575-775, 600-775, 625-775, 650-775, 675-775, 700-775, 725-775, 750-775, 1-750, 25-750, 50-750, 75-750, 100-750, 125-750, 150-750, 175-750, 200-750, 225-750, 250-750, 275-750, 300-750, 325-750, 350-750, 375-750, 400-750, 425-750, 450-750, 475-750, 500-750, 525-750, 550-750, 575-750, 600-750, 625-750, 650-750, 675-750, 700-750, 725-750, 1-725, 25-725, 50-725, 75-725, 100-725, 125-725, 150-725, 175-725, 200-725, 225-725, 250-725, 275-725, 300-725, 325-725, 350-725, 375-725, 400-725, 425-725, 450-725, 475-725, 500-725, 525-725, 550-725, 575-725, 600-725, 625-725, 650-725, 675-725, 700-725, 1-700, 25-700, 50-700, 75-700, 100-700, 125-700, 150-700, 175-700, 200-700, 225-700, 250-700, 275-700, 300-700, 325-700, 350-700, 375-700, 400-700, 425-700, 450-700, 475-700, 500-700, 525-700, 550-700, 575-700, 600-700, 625-700, 650-700, 675-700, 1-675, 25-675, 50-675, 75-675, 100-675, 125-675, 150-675, 175-675, 200-675, 225-675, 250-675, 275-675, 300-675, 325-675, 350-675, 375-675, 400-675, 425-675, 450-675, 475-675, 500-675, 525-675, 550-675, 575-675, 600-675, 625-675, 650-675, 1-650, 25-650, 50-650, 75-650, 100-650, 125-650, 150-650, 175-650, 200-650, 225-650, 250-650, 275-650, 300-650, 325-650, 350-650, 375-650, 400-650, 425-650, 450-650, 475-650, 500-650, 525-650, 550-650, 575-650, 600-650, 625-650, 1-625, 25-625, 50-625, 75-625, 100-625, 125-625, 150-625, 175-625, 200-625, 225-625, 250-625, 275-625, 300-625, 325-625, 350-625, 375-625, 400-625, 425-625, 450-625, 475-625, 500-625, 525-625, 550-625, 575-625,

600-625, 1-600, 25-600, 50-600, 75-600, 100-600, 125-600, 150-600, 175-600, 200-600, 225-600, 250-600, 275-600, 300-600, 325-600, 350-600, 375-600, 400-600, 425-600, 450-600, 475-600, 500-600, 525-600, 550-600, 575-600, 1-575, 25-575, 50-575, 75-575, 100-575, 125-575, 150-575, 175-575, 200-575, 225-575, 250-575, 275-575, 300-575, 325-575, 350-575, 375-575, 400-575, 425-575, 450-575, 475-575, 500-575, 525-575, 550-575, 1-550, 25-550, 50-550, 75-550, 100-550, 125-550, 150-550, 175-550, 200-550, 225-550, 250-550, 275-550, 300-550, 325-550, 350-550, 375-550, 400-550, 425-550, 450-550, 475-550, 500-550, 525-550, 1-525, 25-525, 50-525, 75-525, 100-525, 125-525, 150-525, 175-525, 200-525, 225-525, 250-525, 275-525, 300-525, 325-525, 350-525, 375-525, 400-525, 425-525, 450-525, 475-525, 500-525, 1-500, 25-500, 50-500, 75-500, 100-500, 125-500, 150-500, 175-500, 200-500, 225-500, 250-500, 275-500, 300-500, 325-500, 350-500, 375-500, 400-500, 425-500, 450-500, 475-500, 1-475, 25-475, 50-475, 75-475, 100-475, 125-475, 150-475, 175-475, 200-475, 225-475, 250-475, 275-475, 300-475, 325-475, 350-475, 375-475, 400-475, 425-475, 450-475, 1-450, 25-450, 50-450, 75-450, 100-450, 125-450, 150-450, 175-450, 200-450, 225-450, 250-450, 275-450, 300-450, 325-450, 350-450, 375-450, 400-450, 425-450, 1-425, 25-425, 50-425, 75-425, 100-425, 125-425, 150-425, 175-425, 200-425, 225-425, 250-425, 275-425, 300-425, 325-425, 350-425, 375-425, 400-425, 1-400, 25-400, 50-400, 75-400, 100-400, 125-400, 150-400, 175-400, 200-400, 225-400, 250-400, 275-400, 300-400, 325-400, 350-400, 375-400, 1-375, 25-375, 50-375, 75-375, 100-375, 125-375, 150-375, 175-375, 200-375, 225-375, 250-375, 275-375, 300-375, 325-375, 350-375, 1-350, 25-350, 50-350, 75-350, 100-350, 125-350, 150-350, 175-350, 200-350, 225-350, 250-350, 275-350, 300-350, 325-350, 1-325, 25-325, 50-325, 75-325, 100-325, 125-325, 150-325, 175-325, 200-325, 225-325, 250-325, 275-325, 300-325, 1-300, 25-300, 50-300, 75-300, 100-300, 125-300, 150-300, 175-300, 200-300, 225-300, 250-300, 275-300, 1-275, 25-275, 50-275, 75-275, 100-275, 125-275, 150-275, 175-275, 200-275, 225-275, 250-275, 1-250, 25-250, 50-250, 75-250, 100-250, 125-250, 150-250, 175-250, 200-250, 225-250, 1-225, 25-225, 50-225, 75-225, 100-225, 125-225, 150-225, 175-225, 200-225, 1-200, 25-200, 50-200, 75-200, 100-200, 125-200, 150-200, 175-200, 1-175, 25-175, 50-175, 75-175, 100-175, 125-175, 150-175, 1-150, 25-150, 50-150, 75-150, 100-150, 125-150, 1-125, 25-125, 50-125, 75-125, 100-125, 1-100, 25-100, 50-100, 75-100, 1-75, 25-75, 50-75, 1-50, 25-50, and/or 1-25 of SEQ ID NO:18.



**[0118]** In certain additional preferred embodiments, polynucleotides of the invention comprise, or alternatively, consist of nucleotide residues 400-627, 425-627, 450-627, 475-627, 500-627, 525-627, 550-627, 575-627, 600-627, 400-600, 425-600, 450-600, 475-600, 500-600, 525-600, 550-600, 575-600, 400-575, 425-575, 450-575, 475-575, 500-575, 525-575, 550-575, 400-550, 425-550, 450-550, 475-550, 500-550, 525-550, 400-500, 425-500, 450-500, 475-500, 400-475, 425-475, 450-475, 400-450, 425-450, 571-800, 600-800, 625-800, 650-800, 675-800, 700-800, 725-800, 750-800, 775-800, 571-775, 600-775, 625-775, 650-775, 675-775, 700-775, 725-775, 750-775, 571-750, 600-750, 625-750, 650-750, 675-750, 700-750, 725-750, 571-725, 600-725, 625-725, 650-725, 675-725, 700-725, 571-700, 600-700, 625-700, 650-700, 675-700, 571-675, 600-675, 625-675, 650-675, 571-650, 600-650, 625-650, 571-625, 600-625, and/or 571-600 of SEQ ID NO:1.

**[0119]** In additional preferred embodiments, polynucleotides of the invention comprise, or alternatively, consist of nucleotide residues 147-500, 147-450, 147-400, 147-350, 200-500, 200-450, 200-400, 200-350, 250-500, 250-450, 250-400, 250-350, 300-500, 300-450, 300-400, 300-350, 350-750, 350-700, 350-650, 350-600, 350-550, 400-750, 400-700, 400-650, 400-600, 400-550, 425-750, 425-700, 425-650, 425-600, 425-550, 450-1020, 450-1001, 450-950, 450-900, 450-850, 450-800, 450-775, 500-1001, 500-950, 500-900, 500-850, 500-800, 500-775, 550-1001, 550-950, 550-900, 550-850, 550-800, 550-775, 600-1001, 600-950, 600-900, 600-850, 600-800, 600-775, 650-1001, 650-950, 650-900, 650-850, 650-800, 650-775, 700-1001, 700-950, 700-900, 700-850, 700-800, 700-775, 825-1082, 850-1082, 875-1082, 900-1082, 925-1082, 950-1082, 975-1082, 1000-1082, 1025-1082, and/or 1050-1082 of SEQ ID NO:1.

**[0120]** Preferably, the polynucleotide fragments of the invention encode a polypeptide which demonstrates a Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV functional activity. By a polypeptide demonstrating "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length and/or secreted Neutrokin- $\alpha$  polypeptide and/or Neutrokin- $\alpha$ SV polypeptide. Such functional activities include, but are not limited to, biological activity (e.g., ability to stimulate B cell proliferation, survival, differentiation, and/or activation), antigenicity (ability to bind or compete with a Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide for binding to an anti-Neutrokin- $\alpha$  and/or anti-Neutrokin- $\alpha$ SV

antibody], immunogenicity (ability to generate antibody which binds to a Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide), ability to form multimers (as described below in the "Neutrokin-alpha Polypeptides" section) with Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides of the invention, ability to form heteromultimers (as described below in the "Neutrokin-alpha Polypeptides" section) with APRIL polypeptides (e.g., SEQ ID NO:20 or SEQ ID NO:47; PCT International Publication Number WO97/33902; GenBank Accession No. AF046888 (nucleotide) and AAC6132 (protein); J. Exp. Med. 188(6):1185-1190), ability to bind to a receptor or ligand (e.g., transmembrane activator and CAML interactor (TACI, GenBank accession number AAC51790), and B-cell maturation antigen (BCMA, GenBank accession number NP\_001183)) for a Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide, and ability to stimulate a Neutrokin-alpha and/or Neutrokin-alphaSV receptor signalling cascade (e.g., to activate calcium-modulator and cyclophilin ligand ("CAML"), calcineurin, nuclear factor of activated T cells transcription factor ("NF-AT"), nuclear factor-kappa B ("NF-kappa B"), activator protein-1 (AP-1), SRF, extracellular-signal regulated kinase 1 (ERK-1), polo like kinases (PLK), ELF-1, high mobility group I (HMG-I), and/or high mobility group Y (HMG-Y)).

**[0121]** In additional specific embodiments, the polynucleotide fragments of the invention encode a polypeptide comprising, or alternatively, consisting of the predicted intracellular domain (amino acids 1 to 46 of SEQ ID NO:2), the predicted transmembrane domain (amino acids 47 to 72 of SEQ ID NO:2), the predicted extracellular domain (amino acids 73 to 285 of SEQ ID NO:2), or the predicted TNF conserved domain (amino acids 191 to 284 of SEQ ID NO:2) of Neutrokin-alpha. In additional embodiments, the polynucleotide fragments of the invention encode a polypeptide comprising, or alternatively, consisting of any combination of 1, 2, 3, or all 4 of the above recited domains. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

**[0122]** In additional specific embodiments, the polynucleotide fragments of the invention encode a polypeptide comprising, or alternatively, consisting of the predicted intracellular domain (amino acids 1 to 46 of SEQ ID NO:19), the predicted transmembrane domain (amino acids 47 to 72 of SEQ ID NO:19), the predicted extracellular domain (amino acids 73 to 266 of SEQ ID NO:19), or the predicted TNF

conserved domain (amino acids 172 to 265 of SEQ ID NO:19) of Neutrokin-alphaSV. In additional embodiments, the polynucleotide fragments of the invention encode a polypeptide comprising, or alternatively, consisting of any combination of 1, 2, 3, or all 4 of the above recited domains. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

**[0123]** In another embodiment, polynucleotide fragments of the invention comprise, or alternatively consist of, polynucleotides which encode an amino acid sequence selected from residues Met-1 to Lys-113, Leu-114 to Thr-141, Ile-142 to Lys-160, Gly-161 to Gln-198, Val-199 to Ala-248, and Gly-250 to Leu-285 of SEQ ID NO:2. Moreover, polynucleotides that encode any combination of two, three, four, five or more of these amino acid sequences are also encompassed by the invention. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

**[0124]** In another embodiment, polynucleotide fragments of the invention comprise, or alternatively consist of, polynucleotides which encode an amino acid sequence selected from residues Met-1 to Lys 113, Leu-114 to Thr-141, Gly-142 to Gln-179, Val-180 to Ala-229, and Gly-230 to Leu-266 of SEQ ID NO:19. Moreover, polynucleotides that encode any combination of two, three, four, five or more of these amino acid sequences are also encompassed by the invention. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

**[0125]** In another embodiment, polynucleotide fragments of the invention comprise, or alternatively consist of, polynucleotides which encode an amino acid sequence selected from residues Met-1 to Lys-106, Leu-107 to Thr-134, Glu-135 to Asn-165, Ile-167 to Lys-184, Gly-185 to Gln-224, Val-225 to Ala-272, and Gly-273 to Leu-309 of SEQ ID NO:39. Moreover, polynucleotides that encode any combination of two, three, four, five or more of these amino acid sequences are also encompassed by the invention. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

**[0126]** In another embodiment, polynucleotide fragments of the invention comprise, or alternatively consist of, polynucleotides which encode an amino acid sequence selected from residues Tyr-1 to Lys-47, Leu-48 to Thr-75, Ile-76 to Lys-94, Gly-95 to Gln-132, Val-133 to Ala-182, and Gly-183 to Ala-219 of SEQ ID NO:28. Moreover, polynucleotides that encode any combination of two, three, four, five or more of these

amino acid sequences are also encompassed by the invention. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

[0127] In another embodiment, polynucleotide fragments of the invention comprise, or alternatively consist of, polynucleotides which encode an amino acid sequence selected from residues Tyr-1 to Lys-47, Leu-48 to Thr-75, Ile-76 to Lys-94, Gly-95 to Gln-132, Val-133 to Ala-182, and Gly-183 to Ala-219 of SEQ ID NO:30. Moreover, polynucleotides that encode any combination of two, three, four, five or more of these amino acid sequences are also encompassed by the invention. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

[0128] In another embodiment, the polynucleotides of the invention comprise, or alternatively consist of, the sequence shown in SEQ ID NO:21. The sequence shown as SEQ ID NO:21 encodes a polypeptide consisting of an initiating methionine residue linked to residues Ala-134 through Leu-285 of the Neutrokin- $\alpha$  polypeptide sequence shown as SEQ ID NO:2. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

[0129] In certain additional preferred embodiments, polynucleotides of the invention comprise, or alternatively, consist of nucleotide residues 1-459, 15-459, 30-459, 45-459, 60-459, 75-459, 90-459, 105-459, 120-459, 135-459, 150-459, 165-459, 180-459, 195-459, 210-459, 225-459, 240-459, 255-459, 270-459, 285-459, 300-459, 315-459, 330-459, 345-459, 360-459, 375-459, 390-459, 405-459, 420-459, 435-459, 450-459, 1-450, 15-450, 30-450, 45-450, 60-450, 75-450, 90-450, 105-450, 120-450, 135-450, 150-450, 165-450, 180-450, 195-450, 210-450, 225-450, 240-450, 255-450, 270-450, 285-450, 300-450, 315-450, 330-450, 345-450, 360-450, 375-450, 390-450, 405-450, 420-450, 435-450, 1-435, 15-435, 30-435, 45-435, 60-435, 75-435, 90-435, 105-435, 120-435, 135-435, 150-435, 165-435, 180-435, 195-435, 210-435, 225-435, 240-435, 255-435, 270-435, 285-435, 300-435, 315-435, 330-435, 345-435, 360-435, 375-435, 390-435, 405-435, 420-435, 1-420, 15-420, 30-420, 45-420, 60-420, 75-420, 90-420, 105-420, 120-420, 135-420, 150-420, 165-420, 180-420, 195-420, 210-420, 225-420, 240-420, 255-420, 270-420, 285-420, 300-420, 315-420, 330-420, 345-420, 360-420, 375-420, 390-420, 405-420, 1-405, 15-405, 30-405, 45-405, 60-405, 75-405, 90-405, 105-405, 120-405, 135-405, 150-405, 165-405, 180-405, 195-405, 210-405, 225-405, 240-405, 255-405, 270-405, 285-405, 300-405, 315-405, 330-405, 345-405, 360-405,

375-405, 390-405, 1-390, 15-390, 30-390, 45-390, 60-390, 75-390, 90-390, 105-390, 120-390, 135-390, 150-390, 165-390, 180-390, 195-390, 210-390, 225-390, 240-390, 255-390, 270-390, 285-390, 300-390, 315-390, 330-390, 345-390, 360-390, 375-390, 1-375, 15-375, 30-375, 45-375, 60-375, 75-375, 90-375, 105-375, 120-375, 135-375, 150-375, 165-375, 180-375, 195-375, 210-375, 225-375, 240-375, 255-375, 270-375, 285-375, 300-375, 315-375, 330-375, 345-375, 360-375, 1-360, 15-360, 30-360, 45-360, 60-360, 75-360, 90-360, 105-360, 120-360, 135-360, 150-360, 165-360, 180-360, 195-360, 210-360, 225-360, 240-360, 255-360, 270-360, 285-360, 300-360, 315-360, 330-360, 345-360, 1-345, 15-345, 30-345, 45-345, 60-345, 75-345, 90-345, 105-345, 120-345, 135-345, 150-345, 165-345, 180-345, 195-345, 210-345, 225-345, 240-345, 255-345, 270-345, 285-345, 300-345, 315-345, 330-345, 1-330, 15-330, 30-330, 45-330, 60-330, 75-330, 90-330, 105-330, 120-330, 135-330, 150-330, 165-330, 180-330, 195-330, 210-330, 225-330, 240-330, 255-330, 270-330, 285-330, 300-330, 315-330, 1-315, 15-315, 30-315, 45-315, 60-315, 75-315, 90-315, 105-315, 120-315, 135-315, 150-315, 165-315, 180-315, 195-315, 210-315, 225-315, 240-315, 255-315, 270-315, 285-315, 300-315, 1-300, 15-300, 30-300, 45-300, 60-300, 75-300, 90-300, 105-300, 120-300, 135-300, 150-300, 165-300, 180-300, 195-300, 210-300, 225-300, 240-300, 255-300, 270-300, 285-300, 1-285, 15-285, 30-285, 45-285, 60-285, 75-285, 90-285, 105-285, 120-285, 135-285, 150-285, 165-285, 180-285, 195-285, 210-285, 225-285, 240-285, 255-285, 270-285, 1-270, 15-270, 30-270, 45-270, 60-270, 75-270, 90-270, 105-270, 120-270, 135-270, 150-270, 165-270, 180-270, 195-270, 210-270, 225-270, 240-270, 255-270, 1-255, 15-255, 30-255, 45-255, 60-255, 75-255, 90-255, 105-255, 120-255, 135-255, 150-255, 165-255, 180-255, 195-255, 210-255, 225-255, 240-255, 1-240, 15-240, 30-240, 45-240, 60-240, 75-240, 90-240, 105-240, 120-240, 135-240, 150-240, 165-240, 180-240, 195-240, 210-240, 225-240, 1-225, 15-225, 30-225, 45-225, 60-225, 75-225, 90-225, 105-225, 120-225, 135-225, 150-225, 165-225, 180-225, 195-225, 210-225, 1-210, 15-210, 30-210, 45-210, 60-210, 75-210, 90-210, 105-210, 120-210, 135-210, 150-210, 165-210, 180-210, 195-210, 1-195, 15-195, 30-195, 45-195, 60-195, 75-195, 90-195, 105-195, 120-195, 135-195, 150-195, 165-195, 180-195, 1-180, 15-180, 30-180, 45-180, 60-180, 75-180, 90-180, 105-180, 120-180, 135-180, 150-180, 165-180, 1-165, 15-165, 30-165, 45-165, 60-165, 75-165, 90-165, 105-165, 120-165, 135-165, 150-165, 1-150, 15-150, 30-150, 45-150, 60-150, 75-150, 90-150, 105-150,

120-150, 135-150, 1-135, 15-135, 30-135, 45-135, 60-135, 75-135, 90-135, 105-135, 120-135, 1-120, 15-120, 30-120, 45-120, 60-120, 75-120, 90-120, 105-120, 1-105, 15-105, 30-105, 45-105, 60-105, 75-105, 90-105, 1-90, 15-90, 30-90, 45-90, 60-90, 75-90, 1-75, 15-75, 30-75, 45-75, 60-75, 1-60, 15-60, 30-60, 45-60, 1-45, 15-45, 30-45, 1-30, and/or 15-30 of SEQ ID NO:21. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

[0130] Accordingly, specific embodiments of the invention are directed to polynucleotides encoding polypeptides which comprise, or alternatively consist of, the amino acid sequence of beta pleated sheet region A, A', B, B', C, D, E, F, G, or H disclosed in Figures 7A-1 and 7A-2 and described in Example 6. Additional embodiments of the invention are directed to polynucleotides encoding Neutrokin-alpha polypeptides which comprise, or alternatively consist of, any combination of 1, 2, 3, 4, 5, 6, 7, 8, 9 or all 10 of beta pleated sheet regions A-H disclosed in Figures 7A-1 and 7A-2 and described in Example 6. Additional preferred embodiments of the invention are directed to polypeptides which comprise, or alternatively consist of, the Neutrokin-alpha amino acid sequence of beta pleated sheet region A, A', B, B', C, D, E, F, G, or H disclosed in Figures 7A-1 and 7A-2 and described in Example 6. Additional embodiments of the invention are directed Neutrokin-alpha polypeptides which comprise, or alternatively consist of, any combination of 1, 2, 3, 4, 5, 6, 7, 8, 9 or all 10 of beta pleated sheet regions A through H disclosed in Figures 7A-1 and 7A-2 and described in Example 6.

[0131] In certain other preferred embodiments, polynucleotides of the invention comprise, or alternatively consist of, nucleotide residues 34-57, 118-123, 133-141, 151-159, 175-216, 232-255, 280-315, 328-357, 370-393, and/or 430-456 of SEQ ID NO:21. Polypeptides encoded by these polynucleotides are also encompassed by the invention. These polynucleotide and polypeptide fragments correspond to the predicted beta-pleated sheet regions shown in Figures 7A-1 and 7A-2. In certain embodiments, polynucleotides of the invention comprise, or alternatively consist of, a polynucleotide sequence at least 90%, 95%, 96%, 97%, 98% or 99% identical to the polynucleotide sequence encoding one, two, three, four, five, six, seven, eight, nine or ten of the beta-pleated sheet regions described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these polynucleotide sequences are also encompassed by the invention. In

another embodiment, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to one, two, three, four, five, six, seven, eight, nine or ten of the beta-pleated sheet polynucleotides of the invention described above. The meaning of the phrase "stringent conditions" as used herein is described *infra*.

**[0132]** In further preferred embodiments, polynucleotides of the invention comprise, or alternatively consist of, nucleotide residues 576-599, 660-665, 675-683, 693-701, 717-758, 774-803, 822-857, 870-899, 912-935, and/or 972-998 of SEQ ID NO:1. Polypeptides encoded by these polynucleotide fragments are also encompassed by the invention. These polynucleotide and polypeptide fragments correspond to the predicted beta-pleated sheet regions shown in Figures 7A-1 and 7A-2.

**[0133]** In additional preferred embodiments, polynucleotides of the invention comprise, or alternatively consist of, nucleotide residues 457-462, 472-480, 490-498, 514-555, 571-600, 619-654, 667-696, 699-732, and/or 769-795 of SEQ ID NO:18. Polypeptides encoded by these polynucleotide fragments are also encompassed by the invention. These polynucleotide and polypeptide fragments correspond to the predicted beta-pleated sheet regions shown in Figures 7A-1 and 7A-2.

**[0134]** In yet further preferred embodiments, polynucleotides of the invention comprise, or alternatively consist of, nucleotide residues 124-129, 139-147, 157-165, 181-222, 238-267, 286-321, 334-363, 376-399, and/or 436-462 of SEQ ID NO:22. Polypeptides encoded by these polynucleotide fragments are also encompassed by the invention. These polynucleotide and polypeptide fragments correspond to the predicted beta-pleated sheet regions shown in Figures 7A-1 and 7A-2. Polypeptides comprising, or alternatively, consisting of the amino acid sequence of any combination of one, two, three, four, five, six, seven, eight, nine, ten, or all of these regions are encompassed by the invention.

**[0135]** The relative positions of several intron/exon boundaries were determined for the mouse Neutrokin-alpha (SEQ ID NO:39) based on sequence analysis of mouse genomic DNA. The apparent second exon from the 5' end of the mouse Neutrokin-alpha genomic clone (preliminarily designated "Exon 2") consists of Tyr-187 to Gln-222 of the sequence shown in SEQ ID NO:39. The apparent third exon from the 5' end of the mouse

Neutrokin- $\alpha$  genomic clone (preliminarily designated "Exon 3") comprises Val-223 to Gly-273 of the sequence shown in SEQ ID NO:39.

[0136] Thus, in one embodiment, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues Tyr-187 to Gln-222 of SEQ ID NO:39. The present invention is also directed to nucleic acid molecules comprising, or alternatively, consisting of, a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the polynucleotide sequence encoding the mouse Neutrokin- $\alpha$  polypeptides described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these nucleic acids and/or polynucleotide sequences are also encompassed by the invention.

[0137] In another embodiment, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues Val-223 to Gly-273 of SEQ ID NO:39. The present invention is also directed to nucleic acid molecules comprising, or alternatively, consisting of, a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the polynucleotide sequence encoding the mouse Neutrokin- $\alpha$  polypeptides described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these nucleic acids and/or polynucleotide sequences are also encompassed by the invention.

[0138] Moreover, the relative positions of the corresponding intron/exon boundaries were determined for human Neutrokin- $\alpha$  (SEQ ID NO:1 and SEQ ID NO:2) based on an alignment of the sequences of mouse and human Neutrokin- $\alpha$  polypeptides. The apparent second exon from the 5' end of human Neutrokin- $\alpha$  (also preliminarily designated "Exon 2") consists of, Tyr-163 to Gln-198 of the sequence shown in SEQ ID NO:2. The apparent third exon from the 5' end of human Neutrokin- $\alpha$  (also preliminarily designated "Exon 3") consists of, Val-199 to Gly-249 of the sequence shown in SEQ ID NO:2.

[0139] Thus, in one embodiment, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues Tyr-163 to Gln-198 of SEQ ID NO:2. The present invention is also directed to nucleic acid molecules comprising, or alternatively, consisting of, a polynucleotide



sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the polynucleotide sequence encoding the Neutrokin- $\alpha$  polypeptides described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these nucleic acids and/or polynucleotide sequences are also encompassed by the invention.

**[0140]** In another embodiment, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues Val-199 to Gly-249 of SEQ ID NO:2. The present invention is also directed to nucleic acid molecules comprising, or alternatively, consisting of, a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the polynucleotide sequence encoding the Neutrokin- $\alpha$  polypeptides described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these nucleic acids and/or polynucleotide sequences are also encompassed by the invention. The functional activity of Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods as described herein and as are well known in the art.

**[0141]** For example, in one embodiment where one is assaying for the ability to bind or compete with full-length Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide for binding to anti-Neutrokin- $\alpha$  and/or anti-Neutrokin- $\alpha$ SV antibody or binding to Neutrokin- $\alpha$  receptor(s) and/or Neutrokin- $\alpha$ SV receptor(s) on B cells, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, *in situ* immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled.

Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

**[0142]** In another embodiment, where a Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV ligand is identified, or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky, E., et al., 1995, *Microbiol. Rev.* 59:94-123. In another embodiment, physiological correlates of Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV binding to its substrates (signal transduction) can be assayed.

**[0143]** In addition, assays described herein (see e.g., Examples 6 and 7) and otherwise known in the art may routinely be applied to measure the ability of Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides and fragments, variants derivatives and analogs thereof to elicit Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV related biological activity (e.g., to stimulate, or alternatively to inhibit (in the case of Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV antagonists) signalling mediated by Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV; to stimulate, or alternatively to inhibit B cell proliferation, differentiation and/or activation; and/or to increase or decrease B cell survival *in vitro* or *in vivo*).

**[0144]** Other methods will be known to the skilled artisan and are within the scope of the invention.

**[0145]** In additional embodiments, the polynucleotides of the invention encode polypeptides comprising, or alternatively consisting of, functional attributes of Neutrokin- $\alpha$  and Neutrokin- $\alpha$ SV. Preferred embodiments of the invention in this regard include fragments that comprise, or alternatively consist of,  $\alpha$ -helix and  $\alpha$ -helix forming regions (" $\alpha$ -regions"),  $\beta$ -sheet and  $\beta$ -sheet forming regions (" $\beta$ -regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions,  $\alpha$  amphipathic regions,  $\beta$  amphipathic regions, flexible regions, surface-forming regions and high antigenic index regions of Neutrokin- $\alpha$  and Neutrokin- $\alpha$ SV polypeptides.

[0146] It is believed one or more of the beta pleated sheet regions of Neutrokin- $\alpha$  disclosed in Figures 7A-1 and 7A-2 is important for dimerization and also for interactions between Neutrokin- $\alpha$  and its ligands.

[0147] Certain preferred regions in this regard are set out in Figure 3 (Table I). The data presented in Figure 3 and that presented in Table I, merely present a different format of the same results obtained when the amino acid sequence of SEQ ID NO:2 is analyzed using the default parameters of the DNA\*STAR computer algorithm.

[0148] The above-mentioned preferred regions set out in Figure 3 and in Table I include, but are not limited to, regions of the aforementioned types identified by analysis of the amino acid sequence set out in Figures 1A and 1B. As set out in Figure 3 and in Table I, such preferred regions include Garnier-Robson  $\alpha$ -regions,  $\beta$ -regions, turn-regions, and coil-regions, Chou-Fasman  $\alpha$ -regions,  $\beta$ -regions, and coil-regions, Kyte-Doolittle hydrophilic regions and hydrophobic regions, Eisenberg  $\alpha$ - and  $\beta$ -amphipathic regions, Karplus-Schulz flexible regions, Emini surface-forming regions and Jameson-Wolf regions of high antigenic index. Among highly preferred polynucleotides in this regard are those that encode polypeptides comprising, or alternatively consisting of, regions of Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV that combine several structural features, such as several (e.g., 1, 2, 3 or 4) of the features set out above. Polypeptides encoded by the polynucleotides are also encompassed by the invention.

[0149] Additionally, the data presented in columns VIII, IX, XIII, and XIV of Table I can routinely be used to determine regions of Neutrokin- $\alpha$  which exhibit a high degree of potential for antigenicity (column VIII of Table I represents hydrophilicity according to Kyte-Doolittle; column IX of Table I represents hydrophobicity according to Hopp-Woods; column XIII of Table I represents antigenic index according to Jameson-Wolf; and column XIV of Table I represents surface probability according to Emini). Regions of high antigenicity are determined from the data presented in columns VIII, IX, XIII, and/or IV by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response. The data presented in Figure 6 can also routinely be presented in a similar tabular format by simply examining the amino acid sequence disclosed in Figure 6 (SEQ ID NO:19) using the

modules and algorithms of the DNA\*STAR set on default parameters. As above, the amino acid sequence presented in Figure 6 can also be used to determine regions of Neutrokin-alpha which exhibit a high degree of potential for antigenicity whether presented as a Figure (as in Figure 6) or a table (as in Table I).

**Table I**

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Met	1	A	.	.	.	.	.	.	0.73	-0.71	.	.	.	0.95	1.39
Asp	2	A	.	.	.	.	T	.	1.12	-0.66	*	.	.	1.15	1.56
Asp	3	A	.	.	.	.	T	.	1.62	-1.09	*	.	.	1.15	2.12
Ser	4	A	.	.	.	.	T	.	2.01	-1.51	.	.	.	1.15	4.19
Thr	5	A	.	.	.	.	T	.	2.40	-2.13	.	.	F	1.30	4.35
Glu	6	A	A	.	.	.	.	.	2.70	-1.73	*	*	F	0.90	4.51
Arg	7	A	A	.	.	.	.	.	2.81	-1.34	*	*	F	0.90	4.51
Glu	8	A	A	.	.	.	.	.	2.00	-1.73	*	*	F	0.90	6.12
Gln	9	A	A	.	.	.	.	.	1.99	-1.53	*	*	F	0.90	2.91
Ser	10	A	.	.	B	.	.	.	2.00	-1.04	*	*	F	0.90	2.15
Arg	11	A	.	.	B	.	.	.	1.33	-0.66	*	*	F	0.90	1.66
Leu	12	A	.	.	B	.	.	.	0.41	-0.09	*	*	F	0.45	0.51
Thr	13	A	.	.	B	.	.	.	0.46	0.20	*	*	F	-0.15	0.32
Ser	14	A	A	.	.	.	.	.	0.50	-0.19	*	*	.	0.30	0.32
Cys	15	A	A	.	.	.	.	.	0.91	-0.19	*	*	.	0.30	0.78
Leu	16	A	A	.	.	.	.	.	0.80	-0.87	*	*	F	0.90	1.06
Lys	17	A	A	.	.	.	.	.	1.61	-1.36	.	*	F	0.90	1.37
Lys	18	A	A	.	.	.	.	.	1.32	-1.74	.	*	F	0.90	4.44
Arg	19	A	A	.	.	.	.	.	1.67	-1.70	.	*	F	0.90	5.33
Glu	20	A	A	.	.	.	.	.	1.52	-2.39	.	*	F	0.90	5.33
Glu	21	A	A	.	.	.	.	.	2.38	-1.70	.	*	F	0.90	2.20
Met	22	A	A	.	.	.	.	.	2.33	-1.70	.	*	F	0.90	2.24
Lys	23	A	A	.	.	.	.	.	1.62	-1.70	*	*	F	0.90	2.24
Leu	24	A	A	.	.	.	.	.	0.66	-1.13	*	*	F	0.75	0.69
Lys	25	A	A	.	.	.	.	.	0.36	-0.49	.	*	F	0.45	0.52
Glu	26	A	A	.	B	.	.	.	-0.53	-0.71	*	*	.	0.60	0.35
Cys	27	A	A	.	B	.	.	.	-0.74	-0.03	*	*	.	0.30	0.30
Val	28	A	A	.	B	.	.	.	-1.00	-0.03	*	*	.	0.30	0.12
Ser	29	A	A	.	B	.	.	.	-0.08	0.40	*	*	.	-0.30	0.11
Ile	30	A	.	.	B	.	.	.	-0.08	0.40	*	*	.	-0.30	0.40
Leu	31	A	.	.	B	.	.	.	-0.08	-0.17	*	.	.	0.45	1.08
Pro	32	.	.	.	B	.	.	C	0.29	-0.81	*	.	F	1.10	1.39
Arg	33	.	.	.	.	T	.	.	0.93	-0.81	.	*	F	1.50	2.66
Lys	34	.	.	.	.	T	.	.	0.93	-1.07	.	.	F	1.84	4.98
Glu	35	.	.	.	.	.	.	C	0.97	-1.37	*	*	F	1.98	4.32
Ser	36	.	.	.	.	.	T	C	1.89	-1.16	*	*	F	2.52	1.64
Pro	37	.	.	.	.	.	T	C	1.80	-1.16	*	*	F	2.86	1.60
Ser	38	.	.	.	.	T	T	.	1.39	-0.77	*	.	F	3.40	1.24
Val	39	A	.	.	.	.	T	.	1.39	-0.39	.	*	F	2.36	1.24
Arg	40	A	.	.	.	.	.	.	1.39	-0.77	*	*	F	2.46	1.60

Table I (continued)

Res Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Ser 41	A	.	.	.	.	.	.	1.34	-1.20	*	*	F	2.46	2.00
Ser 42	.	.	.	.	T	T	.	1.60	-1.16	.	*	F	3.06	2.67
Lys 43	.	.	.	.	T	T	.	1.09	-1.80	.	*	F	3.06	2.72
Asp 44	.	.	.	.	T	T	.	1.13	-1.11	*	*	F	3.40	1.67
Gly 45	A	.	.	.	.	T	.	0.43	-0.81	*	*	F	2.66	1.03
Lys 46	A	A	.	.	.	.	.	0.14	-0.70	.	.	F	1.77	0.52
Leu 47	A	A	.	.	.	.	.	0.13	-0.20	*	.	.	0.98	0.31
Leu 48	A	A	.	.	.	.	.	-0.72	0.29	*	.	.	0.04	0.46
Ala 49	A	A	.	.	.	.	.	-1.53	0.54	.	*	.	-0.60	0.19
Ala 50	A	A	.	.	.	.	.	-2.00	1.23	.	.	.	-0.60	0.19
Thr 51	A	A	.	.	.	.	.	-2.63	1.23	.	.	.	-0.60	0.19
Leu 52	A	A	.	.	.	.	.	-2.63	1.04	.	.	.	-0.60	0.19
Leu 53	A	A	.	.	.	.	.	-2.63	1.23	.	.	.	-0.60	0.15
Leu 54	A	A	.	.	.	.	.	-2.34	1.41	.	.	.	-0.60	0.09
Ala 55	A	A	.	.	.	.	.	-2.42	1.31	.	.	.	-0.60	0.14
Leu 56	A	A	.	.	.	.	.	-2.78	1.20	.	.	.	-0.60	0.09
Leu 57	A	.	.	.	.	T	.	-2.78	1.09	.	.	.	-0.20	0.06
Ser 58	A	.	.	.	.	T	.	-2.28	1.09	.	.	.	-0.20	0.05
Cys 59	A	.	.	.	.	T	.	-2.32	1.07	.	.	.	-0.20	0.09
Cys 60	A	.	.	.	.	T	.	-2.59	1.03	.	.	.	-0.20	0.08
Leu 61	.	.	B	B	.	.	.	-2.08	0.99	.	.	.	-0.60	0.04
Thr 62	.	.	B	B	.	.	.	-1.97	0.99	.	.	.	-0.60	0.11
Val 63	.	.	B	B	.	.	.	-1.91	1.20	.	.	.	-0.60	0.17
Val 64	.	.	B	B	.	.	.	-1.24	1.39	.	.	.	-0.60	0.33
Ser 65	.	.	B	B	.	.	.	-1.43	1.10	.	.	.	-0.60	0.40
Phe 66	A	.	.	B	.	.	.	-1.21	1.26	.	.	.	-0.60	0.40
Tyr 67	A	.	.	B	.	.	.	-1.49	1.11	.	.	.	-0.60	0.54
Gln 68	A	.	.	B	.	.	.	-1.44	0.97	.	.	.	-0.60	0.41
Val 69	A	.	.	B	.	.	.	-0.59	1.27	.	.	.	-0.60	0.39
Ala 70	A	.	.	B	.	.	.	-0.63	0.89	.	.	.	-0.60	0.43
Ala 71	A	.	.	B	.	.	.	0.07	0.56	.	*	.	-0.60	0.25
Leu 72	A	.	.	.	.	T	.	-0.50	0.16	.	*	.	0.10	0.55
Gln 73	A	.	.	.	.	T	.	-1.09	0.20	.	.	F	0.25	0.45
Gly 74	A	.	.	.	.	T	.	-0.53	0.20	.	.	F	0.25	0.45
Asp 75	A	.	.	.	.	T	.	-0.76	0.09	.	*	F	0.25	0.73
Leu 76	A	A	.	.	.	.	.	-0.06	0.09	.	*	F	-0.15	0.35
Ala 77	A	A	.	.	.	.	.	0.17	-0.31	.	*	.	0.30	0.69
Ser 78	A	A	.	.	.	.	.	0.17	-0.24	.	*	.	0.30	0.42
Leu 79	A	A	.	.	.	.	.	-0.30	-0.24	.	*	.	0.30	0.88
Arg 80	A	A	.	.	.	.	.	-0.30	-0.24	.	*	.	0.30	0.72

Table I (continued)

Res Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Ala 81	A	A	.	.	.	.	.	0.17	-0.34	.	*	.	0.30	0.93
Glu 82	A	A	.	.	.	.	.	0.72	-0.30	.	*	.	0.45	1.11
Leu 83	A	A	.	.	.	.	.	0.99	-0.49	.	*	.	0.30	0.77
Gln 84	A	A	.	.	.	.	.	1.21	0.01	.	*	.	-0.15	1.04
Gly 85	A	A	.	.	.	.	.	1.10	0.01	*	*	.	-0.30	0.61
His 86	A	A	.	.	.	.	.	1.73	0.01	*	*	.	-0.15	1.27
His 87	A	A	.	.	.	.	.	0.92	-0.67	.	*	.	0.75	1.47
Ala 88	A	A	.	.	.	.	.	1.52	-0.39	.	*	.	0.45	1.22
Glu 89	A	A	.	.	.	.	.	0.93	-0.39	.	.	.	0.45	1.39
Lys 90	A	A	.	.	.	.	.	0.93	-0.39	*	.	F	0.60	1.03
Leu 91	A	.	.	.	.	T	.	0.38	-0.46	*	.	.	0.85	1.01
Pro 92	A	.	.	.	.	T	.	0.07	-0.46	.	.	.	0.70	0.59
Ala 93	A	.	.	.	.	T	.	0.07	-0.03	.	.	.	0.70	0.29
Gly 94	A	.	.	.	.	T	.	-0.14	0.47	.	.	.	-0.20	0.36
Ala 95	A	.	.	.	.	.	.	-0.14	0.21	.	*	.	-0.10	0.36
Gly 96	A	.	.	.	.	.	.	0.08	-0.21	.	.	F	0.65	0.71
Ala 97	A	.	.	.	.	.	.	-0.06	-0.21	.	.	F	0.65	0.72
Pro 98	A	.	.	.	.	.	.	-0.28	-0.21	.	*	F	0.65	0.71
Lys 99	A	A	.	.	.	.	.	0.07	-0.03	.	.	F	0.45	0.59
Ala 100	A	A	.	.	.	.	.	0.66	-0.46	.	.	F	0.60	1.01
Gly 101	A	A	.	.	.	.	.	0.41	-0.96	.	.	F	0.90	1.13
Leu 102	A	A	.	.	.	.	.	0.79	-0.89	.	.	F	0.75	0.57
Glu 103	A	A	.	.	.	.	.	0.41	-0.46	*	.	F	0.45	0.88
Glu 104	A	A	.	.	.	.	.	-0.49	-0.46	*	.	F	0.45	0.89
Ala 105	A	A	.	.	.	.	.	-0.21	-0.24	.	.	.	0.30	0.81
Pro 106	A	A	.	.	.	.	.	-0.46	-0.44	.	.	.	0.30	0.67
Ala 107	A	A	.	.	.	.	.	0.01	0.06	.	.	.	-0.30	0.39
Val 108	A	A	.	.	.	.	.	-0.80	0.49	.	*	.	-0.60	0.38
Thr 109	A	A	.	.	.	.	.	-0.76	0.67	.	*	.	-0.60	0.20
Ala 110	A	A	.	.	.	.	.	-1.06	0.24	*	*	.	-0.30	0.40
Gly 111	A	A	.	.	.	.	.	-1.54	0.43	*	*	.	-0.60	0.38
Leu 112	A	A	.	.	.	.	.	-0.96	0.57	*	*	.	-0.60	0.23
Lys 113	.	A	B	.	.	.	.	-0.31	0.09	*	*	.	-0.30	0.39
Ile 114	.	A	B	.	.	.	.	-0.21	0.01	*	.	.	-0.30	0.61
Phe 115	.	A	B	.	.	.	.	-0.21	0.01	*	.	.	0.15	1.15
Glu 116	.	A	.	.	.	.	C	-0.08	-0.17	*	.	F	1.25	0.58
Pro 117	.	A	.	.	.	.	C	0.39	0.26	*	*	F	1.10	1.28
Pro 118	.	.	.	.	.	.	C	0.34	-0.00	.	.	F	2.20	1.47
Ala 119	.	.	.	.	.	T	C	0.89	-0.79	.	*	F	3.00	1.47
Pro 120	.	.	.	.	.	T	C	1.59	-0.36	.	*	F	2.25	0.94

Table I (continued)

Res Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Gly 121	.	.	.	.	T	T	.	1.29	-0.39	.	*	F	2.15	0.98
Glu 122	.	.	.	.	T	T	.	1.20	-0.43	.	.	F	2.00	1.30
Gly 123	.	.	.	.	.	.	C	1.41	-0.54	.	.	F	1.60	1.12
Asn 124	.	.	.	.	.	T	C	2.00	-0.57	.	.	F	1.50	1.97
Ser 125	.	.	.	.	.	T	C	1.91	-0.60	.	*	F	1.50	1.82
Ser 126	.	.	.	.	.	T	C	2.37	-0.21	.	*	F	1.54	2.47
Gln 127	.	.	.	.	.	T	C	2.37	-0.64	.	*	F	2.18	3.01
Asn 128	.	.	.	.	.	.	C	2.76	-0.64	.	.	F	2.32	3.61
Ser 129	.	.	.	.	.	T	C	2.87	-1.03	.	.	F	2.86	5.39
Arg 130	.	.	.	.	T	T	.	2.58	-1.41	*	.	F	3.40	6.09
Asn 131	.	.	.	.	T	T	.	2.02	-1.31	*	.	F	3.06	3.83
Lys 132	.	.	.	.	T	T	.	2.02	-1.07	*	.	F	2.72	2.12
Arg 133	.	.	.	.	T	.	.	1.68	-1.06	*	.	F	2.18	1.88
Ala 134	.	.	.	.	.	.	C	1.77	-0.63	*	.	F	1.64	1.15
Val 135	.	.	.	.	.	.	C	1.66	-0.60	*	.	F	1.49	0.89
Gln 136	.	.	.	.	.	.	C	1.66	-0.60	*	.	F	1.83	0.79
Gly 137	.	.	.	.	.	T	C	1.30	-0.60	*	.	F	2.52	1.35
Pro 138	.	.	.	.	.	T	C	0.33	-0.61	*	.	F	2.86	2.63
Glu 139	.	.	.	.	T	T	.	0.61	-0.61	*	.	F	3.40	1.13
Glu 140	A	.	.	.	.	T	.	1.47	-0.53	*	.	F	2.66	1.64
Thr 141	A	.	.	.	.	.	.	1.47	-0.56	.	.	F	2.12	1.84
Val 142	A	.	.	.	.	.	.	1.14	-0.99	.	.	F	1.78	1.77
Thr 143	A	.	.	.	.	T	.	0.54	-0.41	.	.	F	1.19	0.55
Gln 144	A	.	.	.	.	T	.	0.54	0.27	*	.	F	0.25	0.31
Asp 145	A	.	.	.	.	T	.	-0.27	0.19	*	.	F	0.25	0.73
Cys 146	A	.	.	.	.	T	.	-0.84	0.23	*	.	.	0.10	0.42
Leu 147	A	A	.	.	.	.	.	-0.58	0.43	*	.	.	-0.60	0.17
Gln 148	A	A	.	.	.	.	.	-0.27	0.53	*	.	.	-0.60	0.10
Leu 149	A	A	.	.	.	.	.	-0.57	0.53	*	*	.	-0.30	0.32
Ile 150	A	A	.	.	.	.	.	-0.57	0.34	*	.	.	0.30	0.52
Ala 151	.	A	.	.	.	.	C	-0.21	-0.34	.	*	.	1.40	0.52
Asp 152	.	.	.	.	T	T	.	0.39	-0.26	.	*	F	2.45	0.91
Ser 153	.	.	.	.	.	T	C	0.08	-0.51	.	.	F	3.00	2.00
Glu 154	.	.	.	.	.	T	C	-0.00	-0.71	.	.	F	2.70	2.86
Thr 155	.	.	.	.	.	T	C	0.89	-0.53	*	.	F	2.40	1.20
Pro 156	.	.	.	B	.	.	C	1.52	-0.13	*	.	F	1.56	1.55
Thr 157	.	.	.	B	T	.	.	1.18	-0.51	*	.	F	1.92	1.79
Ile 158	A	.	.	B	.	.	.	1.18	-0.09	.	.	F	1.08	1.23
Gln 159	.	.	.	.	T	T	.	0.93	-0.19	.	.	F	2.04	1.07
Lys 160	.	.	.	.	T	T	.	0.93	0.14	*	.	F	1.60	1.16



**Table I (continued)**

Res Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Gly 161	.	.	.	.	T	T	.	0.44	0.14	*	.	F	1.44	2.38
Ser 162	.	.	.	.	T	T	.	-0.10	0.24	*	.	F	1.28	1.19
Tyr 163	.	.	.	B	T	.	.	0.58	0.49	*	.	.	0.12	0.44
Thr 164	.	.	B	B	.	.	.	0.29	0.91	*	.	.	-0.44	0.69
Phe 165	.	.	B	B	.	.	.	-0.57	1.40	*	.	.	-0.60	0.54
Val 166	.	.	B	B	.	.	.	-1.03	1.70	.	.	.	-0.60	0.29
Pro 167	.	.	B	B	.	.	.	-1.03	1.63	.	.	.	-0.60	0.16
Trp 168	A	.	.	B	.	.	.	-1.49	1.53	.	*	.	-0.60	0.25
Leu 169	A	.	.	B	.	.	.	-1.13	1.53	*	.	.	-0.60	0.29
Leu 170	A	.	.	B	.	.	.	-0.32	0.89	*	.	.	-0.30	0.38
Ter 171	A	.	.	.	.	.	.	0.19	0.46	*	.	.	0.20	0.71
Phe 172	.	.	.	.	T	.	.	0.10	-0.03	*	.	.	1.80	0.85
Lys 173	.	.	.	.	T	T	.	-0.20	-0.33	*	.	F	2.60	1.38
Arg 174	.	.	.	.	.	T	C	-0.20	-0.51	.	.	F	3.00	1.04
Gly 175	.	.	.	.	.	T	C	0.61	-0.21	.	.	F	2.25	0.99
Ser 176	A	.	.	.	.	T	.	0.91	-1.00	*	.	F	2.05	0.86
Ala 177	A	A	.	.	.	.	.	1.66	-1.00	*	.	F	1.35	0.76
Leu 178	A	A	.	.	.	.	.	1.61	-1.00	.	.	F	1.20	1.54
Glu 179	A	A	.	.	.	.	.	1.50	-1.43	.	.	F	0.90	1.98
Glu 180	A	A	.	.	.	.	.	1.89	-1.41	*	.	F	0.90	3.16
Lys 181	A	A	.	.	.	.	.	1.30	-1.91	*	.	F	0.90	7.66
Glu 182	A	A	.	.	.	.	.	1.08	-1.91	.	.	F	0.90	3.10
Asn 183	A	A	.	.	.	.	.	1.03	-1.23	*	*	F	0.90	1.48
Lys 184	A	A	.	.	.	.	.	1.08	-0.59	*	.	F	0.75	0.55
Ile 185	A	A	.	.	.	.	.	1.08	-0.59	*	*	.	0.60	0.63
Leu 186	A	A	.	.	.	.	.	0.72	-0.59	*	*	.	0.60	0.68
Val 187	A	A	.	.	.	.	.	0.38	-0.50	.	*	.	0.30	0.49
Lys 188	A	A	.	.	.	.	.	0.13	-0.07	*	*	F	0.45	0.69
Glu 189	A	.	.	.	.	T	.	-0.61	0.00	*	*	F	0.40	1.32
Thr 190	.	.	.	.	T	T	.	-0.42	0.10	.	*	F	0.80	1.54
Gly 191	.	.	.	.	T	T	.	-0.50	0.24	*	.	F	0.65	0.67
Tyr 192	.	.	.	.	T	T	.	0.11	0.93	*	*	.	0.20	0.27
Phe 193	.	.	B	B	.	.	.	-0.28	1.69	.	.	.	-0.60	0.29
Phe 194	.	.	B	B	.	.	.	-0.28	1.63	.	*	.	-0.60	0.29
Ile 195	.	.	B	B	.	.	.	-0.82	1.60	.	.	.	-0.60	0.32
Tyr 196	.	.	B	B	.	.	.	-1.29	1.49	.	.	.	-0.60	0.28
Gly 197	.	.	.	B	T	.	.	-1.29	1.39	.	.	.	-0.20	0.26
Gln 198	.	.	.	B	T	.	.	-0.90	1.36	.	.	.	-0.20	0.59
Val 199	.	.	.	B	.	.	C	-0.20	1.16	.	.	.	-0.40	0.54
Leu 200	.	.	.	B	.	.	C	0.73	0.40	.	.	.	-0.10	0.92

**Table I (continued)**

Res Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Tyr 201	.	.	.	.	T	T	.	0.67	-0.03	.	.	.	1.25	1.06
Thr 202	.	.	.	.	T	T	.	0.77	0.06	.	.	F	0.80	2.06
Asp 203	.	.	.	.	T	T	.	0.18	0.17	.	.	F	0.80	3.91
Lys 204	A	.	.	.	.	T	.	0.43	-0.01	.	.	F	1.00	2.52
Thr 205	A	A	.	.	.	.	.	0.90	-0.16	.	.	F	0.60	1.73
Tyr 206	A	A	.	.	.	.	.	1.11	-0.21	.	.	.	0.45	1.03
Ala 207	A	A	.	.	.	.	.	0.61	0.29	.	.	.	-0.30	0.70
Met 208	A	A	.	.	.	.	.	-0.28	0.97	.	.	.	-0.60	0.40
Gly 209	A	A	.	B	.	.	.	-0.32	1.17	*	.	.	-0.60	0.18
His 210	A	A	.	B	.	.	.	0.10	0.81	*	.	.	-0.60	0.31
Leu 211	A	A	.	B	.	.	.	0.39	0.31	.	.	.	-0.30	0.61
Ile 212	A	A	.	B	.	.	.	1.02	-0.30	.	.	.	0.45	1.22
Gln 213	A	A	.	B	.	.	.	0.77	-0.73	.	*	.	0.75	1.80
Arg 214	A	A	.	B	.	.	.	1.08	-0.59	.	*	F	0.90	1.62
Lys 215	A	A	.	B	.	.	.	0.26	-0.77	*	*	F	0.90	3.14
Lys 216	A	A	.	B	.	.	.	0.37	-0.81	.	*	F	0.90	1.35
Val 217	.	A	B	B	.	.	.	0.91	-0.43	*	*	.	0.30	0.60
His 218	.	A	B	B	.	.	.	0.91	-0.00	.	*	.	0.30	0.29
Val 219	.	A	B	B	.	.	.	0.80	-0.00	*	*	.	0.30	0.25
Phe 220	.	.	B	B	.	.	.	-0.06	-0.00	*	.	.	0.30	0.57
Gly 221	A	.	.	B	.	.	.	-0.40	0.04	.	*	.	-0.30	0.35
Asp 222	A	.	.	.	.	.	.	-0.36	-0.07	*	.	.	0.50	0.63
Glu 223	A	.	.	.	.	.	.	-1.18	-0.03	*	.	.	0.50	0.60
Leu 224	A	.	.	B	.	.	.	-0.63	-0.17	.	.	.	0.30	0.45
Ser 225	A	.	.	B	.	.	.	-0.74	-0.11	.	.	.	0.30	0.39
Leu 226	A	.	.	B	.	.	.	-1.10	0.57	.	*	.	-0.60	0.18
Val 227	A	.	.	B	.	.	.	-0.99	1.36	.	*	.	-0.60	0.19
Thr 228	A	.	.	B	.	.	.	-1.66	0.67	*	*	.	-0.60	0.28
Leu 229	A	.	.	B	.	.	.	-1.73	0.86	*	.	.	-0.60	0.18
Phe 230	A	.	.	B	.	.	.	-1.43	0.86	*	.	.	-0.60	0.17
Arg 231	A	.	.	B	.	.	.	-0.62	0.61	*	.	.	-0.60	0.21
Cys 232	.	.	.	B	T	.	.	-0.37	0.53	*	.	.	-0.20	0.41
Ile 233	.	.	.	B	T	.	.	-0.27	0.46	*	.	.	-0.20	0.46
Gln 234	.	.	.	B	T	.	.	0.54	0.10	*	.	.	0.10	0.37
Asn 235	.	.	.	B	.	.	C	0.93	0.10	*	.	.	0.05	1.19
Met 236	.	.	.	B	.	.	C	0.01	0.01	*	.	F	0.20	2.44
Pro 237	.	.	.	B	.	.	C	0.47	0.01	*	.	F	0.44	1.16
Glu 238	.	.	.	.	T	.	.	1.36	0.04	*	.	F	1.08	1.12
Thr 239	.	.	.	.	.	.	C	1.36	0.04	*	.	F	1.12	1.82
Leu 240	.	.	.	.	.	.	C	1.06	-0.17	*	.	F	1.96	1.89

Table I (continued)

Res Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Pro 241	.	.	.	.	T	.	.	0.99	-0.21	.	.	F	2.40	1.46
Asn 242	.	.	.	.	T	.	.	0.96	0.36	.	.	F	1.41	0.54
Asn 243	.	.	.	.	T	T	.	0.66	0.63	.	.	F	1.22	1.03
Ser 244	.	.	.	.	T	T	.	0.38	0.33	.	.	F	1.13	0.89
Cys 245	.	.	.	.	T	T	.	0.84	0.40	.	.	.	0.74	0.56
Tyr 246	.	.	.	.	T	T	.	0.17	0.43	.	.	.	0.20	0.35
Ser 247	A	.	.	.	.	.	.	-0.42	0.71	.	.	.	-0.40	0.18
Ala 248	A	A	.	.	.	.	.	-0.38	0.83	.	.	.	-0.60	0.34
Gly 249	A	A	.	.	.	.	.	-0.89	0.26	.	.	.	-0.30	0.43
Ile 250	A	A	.	.	.	.	.	-0.22	0.19	*	.	.	-0.30	0.27
Ala 251	A	A	.	.	.	.	.	0.02	-0.20	*	.	.	0.30	0.46
Lys 252	A	A	.	.	.	.	.	-0.02	-0.70	.	.	.	0.60	0.80
Leu 253	A	A	.	.	.	.	.	0.57	-0.70	.	.	F	0.90	1.13
Glu 254	A	A	.	.	.	.	.	0.91	-1.39	.	.	F	0.90	1.87
Glu 255	A	A	.	.	.	.	.	0.99	-1.89	.	.	F	0.90	1.62
Gly 256	A	A	.	.	.	.	.	1.58	-1.20	.	*	F	0.90	1.62
Asp 257	A	A	.	.	.	.	.	0.72	-1.49	.	*	F	0.90	1.62
Glu 258	A	A	.	.	.	.	.	0.94	-0.80	*	*	F	0.75	0.77
Leu 259	A	A	.	.	.	.	.	0.06	-0.30	*	*	.	0.30	0.79
Gln 260	A	A	.	.	.	.	.	-0.16	-0.04	*	.	.	0.30	0.33
Leu 261	A	A	.	.	.	.	.	0.30	0.39	*	.	.	-0.30	0.30
Ala 262	A	A	.	.	.	.	.	0.30	0.39	*	.	.	-0.30	0.70
Ile 263	A	A	.	.	.	.	.	0.30	-0.30	.	*	.	0.30	0.70
Pro 264	A	.	.	.	.	T	.	0.52	-0.30	.	*	F	1.00	1.37
Arg 265	A	.	.	.	.	T	.	0.52	-0.49	.	*	F	1.00	1.37
Glu 266	A	.	.	.	.	T	.	0.44	-0.59	*	*	F	1.30	3.38
Asn 267	A	.	.	.	.	T	.	0.73	-0.59	*	*	F	1.30	1.53
Ala 268	A	.	.	.	.	.	.	0.81	-0.63	*	*	.	0.95	1.05
Gln 269	A	.	.	.	.	.	.	1.02	0.06	*	*	.	-0.10	0.50
Ile 270	A	.	.	.	.	.	.	0.57	0.06	.	*	.	0.15	0.52
Ser 271	.	.	.	.	.	.	C	0.57	0.09	.	*	.	0.60	0.51
Leu 272	.	.	.	.	.	.	C	-0.29	-0.41	.	*	F	1.60	0.49
Asp 273	.	.	.	.	T	T	.	-0.01	-0.17	.	*	F	2.25	0.52
Gly 274	.	.	.	.	T	T	.	-0.71	-0.37	.	*	F	2.50	0.56
Asp 275	.	.	.	.	T	T	.	-0.52	0.03	.	*	F	1.65	0.59
Val 276	A	.	.	.	.	T	.	-0.57	0.13	.	*	F	1.00	0.30
Thr 277	A	.	.	B	.	.	.	-0.34	0.56	.	*	.	-0.10	0.30
Phe 278	A	.	.	B	.	.	.	-1.16	0.63	.	*	.	-0.35	0.18
Phe 279	A	.	.	B	.	.	.	-0.77	1.31	.	*	.	-0.60	0.20
Gly 280	A	A	.	.	.	.	.	-1.58	0.67	.	*	.	-0.60	0.28

**Table I (continued)**

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Ala	281	A	A	.	.	.	.	.	-1.53	0.87	.	*	.	-0.60	0.27
Leu	282	A	A	.	.	.	.	.	-1.61	0.77	*	.	.	-0.60	0.26
Lys	283	A	A	.	.	.	.	.	-1.30	0.41	*	.	.	-0.60	0.33
Leu	284	A	A	.	.	.	.	.	-0.99	0.41	.	.	.	-0.60	0.42
Leu	285	A	A	.	.	.	.	.	-1.03	0.34	*	.	.	-0.30	0.65

[0150] Additional preferred nucleic acid fragments of the present invention include nucleic acid molecules comprising, or alternatively, consisting of a sequence encoding one or more epitope-bearing portions of Neutrokin- $\alpha$ . In particular, such nucleic acid fragments of the present invention include nucleic acid molecules comprising, or alternatively consisting of, a sequence encoding a polypeptide selected from: from about Phe-115 to about Leu-147, from about Ile-150 to about Tyr-163, from about Ser-171 to about Phe-194, from about Glu-223 to about Tyr-246, and from about Ser-271 to about Phe-278, of the amino acid sequence of SEQ ID NO:2. In this context, "about" means the particularly recited ranges and ranges larger or smaller by several, a few, 5, 4, 3, 2 or 1 amino acid residues at either or both the amino- and carboxy-termini. Polypeptides encoded by these nucleic acid molecules are also encompassed by the invention. Polypeptide fragments which bear antigenic epitopes of the Neutrokin- $\alpha$  may be easily determined by one of skill in the art using the above-described analysis of the Jameson-Wolf antigenic index, as shown in Figure 3. Methods for determining other such epitope-bearing portions of Neutrokin- $\alpha$  are described in detail below.

[0151] Additional preferred nucleic acid fragments of the present invention include nucleic acid molecules comprising, or alternatively consisting of a sequence encoding one or more epitope-bearing portions of Neutrokin- $\alpha$ SV. In particular, such nucleic acid fragments of the present invention include nucleic acid molecules comprising, or alternatively consisting of a sequence encoding a polypeptide selected from about Pro-32 to about Leu-47, from about Glu-116 to about Ser-143, from about Phe-153 to about Tyr-173, from about Pro-218 to about Tyr-227, from about Ser-252 to about Thr-258, from about Ala-232 to about Gln-241; from about Ile-244 to about Ala-249; and from about Ser-252 to about Val-257, of the amino acid sequence of SEQ ID NO:19. In this context, "about" means the particularly recited ranges and ranges larger or smaller by several, a few, 5, 4, 3, 2 or 1 amino acid residues at either or both the amino- and carboxy-termini. Polypeptides encoded by these nucleic acid molecules are also encompassed by the invention. Polypeptide fragments which bear antigenic epitopes of the Neutrokin- $\alpha$  may be easily determined by one of skill in the art using the above-described analysis of the Jameson-Wolf antigenic index. Methods for determining other such epitope-bearing portions of Neutrokin- $\alpha$ SV are described in detail below.

**[0152]** In specific embodiments, the polynucleotides of the invention are less than 100,000 kb, 50,000 kb, 10,000 kb, 1,000 kb, 500 kb, 400 kb, 350 kb, 300 kb, 250 kb, 200 kb, 175 kb, 150 kb, 125 kb, 100 kb, 75 kb, 50 kb, 40 kb, 30 kb, 25 kb, 20 kb, 15 kb, 10 kb, 7.5 kb, or 5 kb in length.

**[0153]** In further embodiments, polynucleotides of the invention comprise at least 15, at least 30, at least 50, at least 100, or at least 250, at least 500, or at least 1000 contiguous nucleotides of Neutrokin- $\alpha$  coding sequence, but consist of less than or equal to 1000 kb, 500 kb, 250 kb, 200 kb, 150 kb, 100 kb, 75 kb, 50 kb, 30 kb, 25 kb, 20 kb, 15 kb, 10 kb, or 5 kb of genomic DNA that flanks the 5' or 3' coding nucleotide set forth in Figures 1A and 1B (SEQ ID NO:1) or Figures 5A and 5B (SEQ ID NO:18). In further embodiments, polynucleotides of the invention comprise at least 15, at least 30, at least 50, at least 100, or at least 250, at least 500, or at least 1000 contiguous nucleotides of Neutrokin- $\alpha$  coding sequence, but do not comprise all or a portion of any Neutrokin- $\alpha$  intron. In another embodiment, the nucleic acid comprising Neutrokin- $\alpha$  coding sequence does not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the Neutrokin- $\alpha$  gene in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

**[0154]** In another embodiment, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a portion of the polynucleotide in a nucleic acid molecule of the invention described above, for instance, the sequence complementary to the coding and/or noncoding sequence depicted in Figures 1A and 1B (SEQ ID NO:1), the sequence of the cDNA clone contained in the deposit having ATCC accession no. 97768, the sequence complementary to the coding sequence and/or noncoding sequence depicted in Figures 5A and 5B (SEQ ID NO:18), the sequence of the cDNA clone contained in the deposit having ATCC accession no. 203518, the sequence complementary to the coding sequence and/or noncoding sequence (i.e., transcribed, untranslated) depicted in SEQ ID NO:21, the sequence complementary to the coding sequence and/or noncoding sequence depicted in SEQ ID NO:22, the sequence complementary to the coding sequence and/or noncoding sequence depicted in SEQ ID NO:27, the sequence complementary to the coding sequence

and/or noncoding sequence depicted in SEQ ID NO:29, the sequence complementary to the coding sequence and/or noncoding sequence depicted in SEQ ID NO:37, or fragments (such as, for example, the open reading frame or a fragment thereof) of these sequences, as described herein. By "stringent hybridization conditions" is intended overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

**[0155]** By a polynucleotide which hybridizes to a "portion" of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides (nt), and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably about 30-70 (e.g., 40, 50, or 60) nucleotides, and even more preferably about any integer in the range of 30-70 or 80-150 nucleotides, or the entire length of the reference polynucleotide. These have uses, which include, but are not limited to, diagnostic probes and primers as discussed above and in more detail below. By a portion of a polynucleotide of "at least about 20 nt in length," for example, is intended to include the particularly recited ranges, larger or smaller by several (i.e. 5, 4, 3, 2, 1, or 0) amino acids, at either extreme or at both extremes of the nucleotide sequence of the reference polynucleotide (e.g., the sequence of one or both of the deposited cDNAs, the complementary strand of the nucleotide sequence shown in Figures 1A and 1B (SEQ ID NO:1), the complementary strand of the nucleotide sequence shown in Figures 5A and 5B (SEQ ID NO:18), the complementary strand of the nucleotide sequence shown in SEQ ID NO:21, the complementary strand of the nucleotide sequence shown in SEQ ID NO:22, the complementary strand of the nucleotide sequence shown in SEQ ID NO:27, the complementary strand of the nucleotide sequence shown in SEQ ID NO:29, and/or the complementary strand of the nucleotide sequence shown in SEQ ID NO:37). Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3' terminal poly (A) tract of the Neutrokin- $\alpha$  cDNA shown in Figures 1A and 1B (SEQ ID NO:1), the 3' terminal poly(A) tract of the Neutrokin- $\alpha$ SV cDNA shown in Figures 5A and 5B (SEQ ID NO:18) or the 3' terminal poly(A) tract of the Neutrokin- $\alpha$ SV cDNA shown in SEQ ID NO:22), or to a complementary stretch of T (or U) residues, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic

acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using oligo dT as a primer).

**[0156]** As indicated, nucleic acid molecules of the present invention which encode a Neutrokin- $\alpha$  polypeptide or a Neutrokin- $\alpha$ SV polypeptide may include, but are not limited to, polynucleotides encoding the amino acid sequence of the respective extracellular domains of the polypeptides, by themselves; and the coding sequence for the extracellular domains of the respective polypeptides and additional sequences, such as those encoding the intracellular and transmembrane domain sequences, or a pre-, or pro- or prepro- protein sequence; the coding sequence of the respective extracellular domains of the polypeptides, with or without the aforementioned additional coding sequences.

**[0157]** Also encoded by nucleic acids of the invention are the above protein sequences together with additional, non-coding sequences, including for example, but not limited to, introns and non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals, for example, ribosome binding and stability of mRNA; an additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities.

**[0158]** Thus, the sequence encoding the polypeptide may be fused to a marker sequence, such as a sequence encoding a peptide which facilitates purification of the fused polypeptide. In certain preferred embodiments of this embodiment of the invention, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., *Proc. Natl. Acad. Sci. USA* 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. The "HA" tag is another peptide useful for purification which corresponds to an epitope derived from the influenza hemagglutinin protein, which has been described by Wilson et al., *Cell* 37: 767 (1984). As discussed below, other such fusion proteins include the Neutrokin- $\alpha$  or the Neutrokin- $\alpha$ SV polypeptides fused to Fc at the N- or C-terminus.

**[0159]** The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode portions, analogs or derivatives of the



Neutrokin- $\alpha$  or Neutrokin- $\alpha$ SV polypeptides of SEQ ID NO:2. Variants may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. *Genes II*, Lewin, B., ed., John Wiley & Sons, New York (1985). Non-naturally occurring variants may be produced using art-known mutagenesis techniques, which include, but are not limited to oligonucleotide mediated mutagenesis, alanine scanning, PCR mutagenesis, site directed mutagenesis (see e.g., Carter et al., Nucl. Acids Res. 13:4331 (1986); and Zoller et al., Nucl. Acids Res. 10:6487 (1982)), cassette mutagenesis (see e.g., Wells et al., *Gene* 34:315 (1985)), restriction selection mutagenesis (see e.g., Wells et al., Philos. Trans. R. Soc. London SerA 317:415 (1986)).

**[0160]** Such variants include those produced by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding regions, non-coding regions, or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides or portions thereof. Also especially preferred in this regard are conservative substitutions.

**[0161]** Additional embodiments of the invention are directed to isolated nucleic acid molecules comprising a polynucleotide which encodes the amino acid sequence of a Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide (e.g., a Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide fragment described herein) having an amino acid sequence which contains at least one conservative amino acid substitution, but not more than 50 conservative amino acid substitutions, even more preferably, not more than 40 conservative amino acid substitutions, still more preferably, not more than 30 conservative amino acid substitutions, and still even more preferably, not more than 20 conservative amino acid substitutions, 10-20 conservative amino acid substitutions, 5-10 conservative amino acid substitutions, 1-5 conservative amino acid substitutions, 3-5 conservative amino acid substitutions, or 1-3 conservative amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a polynucleotide which encodes the amino acid sequence of a Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide to

have an amino acid sequence which contains not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 conservative amino acid substitutions.

[0162] Further embodiments include an isolated nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide having a nucleotide sequence at least 80%, 85%, or 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical to a polynucleotide selected from the group consisting of: (a) a nucleotide sequence encoding the Neutrokin- $\alpha$  polypeptide having the complete amino acid sequence in Figures 1A and 1B (i.e., positions 1 to 285 of SEQ ID NO:2); (b) a nucleotide sequence encoding the Neutrokin- $\alpha$  polypeptide having the complete amino acid sequence in SEQ ID NO:2 excepting the N-terminal methionine (i.e., positions 2 to 285 of SEQ ID NO:2); (c) a fragment of the polypeptide of (b) having Neutrokin- $\alpha$  functional activity (e.g., antigenic or biological activity); (d) a nucleotide sequence encoding the predicted extracellular domain of the Neutrokin- $\alpha$  polypeptide having the amino acid sequence at positions 73-285 in Figures 1A and 1B (SEQ ID NO:2); (e) a nucleotide sequence encoding the Neutrokin- $\alpha$  polypeptide having the amino acid sequence at positions 134-285 in Figures 1A and 1B (SEQ ID NO:2); (f) a nucleotide sequence encoding the Neutrokin- $\alpha$  polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in the deposit having ATCC accession number 97768; (g) a nucleotide sequence encoding the extracellular domain of the Neutrokin- $\alpha$  polypeptide having the amino acid sequence encoded by the cDNA contained in the deposit having ATCC accession number 97768; and (h) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), or (h) above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these polynucleotides and nucleic acid molecules are also encompassed by the invention.

[0163] Highly preferred embodiments of the invention are directed to nucleic acid molecules comprising, or alternatively consisting of a polynucleotide having a nucleotide sequence at least 80%, 85%, 90% identical and more preferably at least 95%, 96%, 97%, 98%, 99% or 100% identical to a polynucleotide sequence encoding the Neutrokin- $\alpha$  polypeptide having the amino acid sequence at positions 134-285 in Figures 1A and 1B (SEQ ID NO:2). Preferred embodiments of the invention are directed to nucleic acid molecules comprising, or alternatively consisting of a polynucleotide having a nucleotide

sequence at least 90% identical to a polynucleotide sequence encoding the Neutrokin-alpha polypeptide having the amino acid sequence at positions 134-285 in Figures 1A and 1B (SEQ ID NO:2). More preferred embodiments of the invention are directed to nucleic acid molecules comprising, or alternatively consisting of a polynucleotide having a nucleotide sequence at least 95% identical to a polynucleotide sequence encoding the Neutrokin-alpha polypeptide having the amino acid sequence at positions 134-285 in Figures 1A and 1B (SEQ ID NO:2). More preferred embodiments of the invention are directed to nucleic acid molecules comprising, or alternatively consisting of a polynucleotide having a nucleotide sequence at least 96% identical to a polynucleotide sequence encoding the Neutrokin-alpha polypeptide having the amino acid sequence at positions 134-285 in Figures 1A and 1B (SEQ ID NO:2).

**[0164]** Additionally, more preferred embodiments of the invention are directed to nucleic acid molecules comprising, or alternatively consisting of a polynucleotide having a nucleotide sequence at least 97% to a polynucleotide sequence encoding the Neutrokin-alpha polypeptide having the amino acid sequence at positions 134-285 in Figures 1A and 1B (SEQ ID NO:2). Additionally, more preferred embodiments of the invention are directed to nucleic acid molecules comprising, or alternatively consisting of a polynucleotide having a nucleotide sequence at least 98% to a polynucleotide sequence encoding the Neutrokin-alpha polypeptide having the amino acid sequence at positions 134-285 in Figures 1A and 1B (SEQ ID NO:2). Additionally, more preferred embodiments of the invention are directed to nucleic acid molecules comprising, or alternatively consisting of a polynucleotide having a nucleotide sequence at least 99% identical to a polynucleotide sequence encoding the Neutrokin-alpha polypeptide having the amino acid sequence at positions 134-285 in Figures 1A and 1B (SEQ ID NO:2).

**[0165]** A further embodiment of the invention relates to an isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of a Neutrokin-alphaSV polypeptide (e.g., a Neutrokin-alphaSV polypeptide fragment described herein) having an amino acid sequence which contains at least one conservative amino acid substitution, but not more than 50 conservative amino acid substitutions, even more preferably, not more than 40 conservative amino acid substitutions, still more preferably not more than 30 conservative amino acid substitutions, and still even more preferably not more than 20 conservative amino acid substitutions. Of course, in order of

ever-increasing preference, it is highly preferable for a polynucleotide which encodes the amino acid sequence of a Neutrokin- $\alpha$  polypeptide to have an amino acid sequence which contains not more than 7-10, 5-10, 3-7, 3-5, 2-5, 1-5, 1-3, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 conservative amino acid substitutions.

[0166] Further embodiments include an isolated nucleic acid molecule comprising, or alternatively, consisting of a polynucleotide having a nucleotide sequence at least 80%, 85% or 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical to a polynucleotide selected from the group consisting of: (a) a nucleotide sequence encoding the Neutrokin- $\alpha$ SV polypeptide having the complete amino acid sequence in Figures 5A and 5B (i.e., positions 1 to 266 of SEQ ID NO:19); (b) a nucleotide sequence encoding the Neutrokin- $\alpha$ SV polypeptide having the complete amino acid sequence in SEQ ID NO:19 excepting the N-terminal methionine (i.e., positions 2 to 266 of SEQ ID NO:2); (c) a nucleotide sequence encoding the predicted extracellular domain of the Neutrokin- $\alpha$ SV polypeptide having the amino acid sequence at positions 73-266 in Figures 5A and 5B (SEQ ID NO:19); (d) a nucleotide sequence encoding the Neutrokin- $\alpha$ SV polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in the deposit having ATCC accession number 203518; (e) a nucleotide sequence encoding the extracellular domain of the Neutrokin- $\alpha$ SV polypeptide having the amino acid sequence encoded by the cDNA clone contained in the deposit having ATCC accession number 203518; and (f) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d) or (e), above.

[0167] Further, the invention includes a polynucleotide comprising, or alternatively, consisting of, a sequence at least 90%, or at least 95%, identical to any portion of at least about 10 contiguous nucleotides, about 20 contiguous nucleotides, about 25 contiguous nucleotides, or about 30 contiguous nucleotides, preferably at least about 40 nucleotides, or at least about 50 nucleotides, of the sequence from nucleotide 1 to nucleotide 1082 in Figures 1A and 1B (SEQ ID NO:1), preferably excluding the nucleotide sequences determined from the above-listed 4 cDNA clones and the nucleotide sequences from nucleotide 797 to 1082, 810 to 1082, and 346 to 542. The invention also includes a polynucleotide comprising, or alternatively consisting of, a sequence at least 90%, or at least 95%, identical to any portion of at least about 10 contiguous nucleotides, about 20 contiguous nucleotides, about 25 contiguous nucleotides, or about 30 contiguous

nucleotides, preferably at least about 40 nucleotides, or at least about 50 nucleotides, of the sequence in Figures 5A and 5B (SEQ ID NO:18), preferably excluding the nucleotide sequences determined from the above-listed 4 cDNA clones. The invention also includes a polynucleotide comprising, or alternatively consisting of a sequence at least 90%, or at least 95%, identical to any portion of at least about 10 contiguous nucleotides, about 20 contiguous nucleotides, about 25 contiguous nucleotides, or about 30 contiguous nucleotides, preferably at least about 40 nucleotides, or at least about 50 nucleotides, of the sequence in SEQ ID NO:21, preferably excluding the nucleotide sequences determined from the above-listed 4 cDNA clones. The invention also includes a polynucleotide comprising a sequence at least 90%, or at least 95%, identical to any portion of at least about 10 contiguous nucleotides, about 20 contiguous nucleotides, about 25 contiguous nucleotides, or about 30 contiguous nucleotides, preferably at least about 40 nucleotides, or at least about 50 nucleotides, of the sequence in SEQ ID NO:22, preferably excluding the nucleotide sequences determined from the above-listed 4 cDNA clones. The invention also includes a polynucleotide comprising a sequence at least 90%, or at least 95%, identical to any portion of at least about 10 contiguous nucleotides, about 20 contiguous nucleotides, about 25 contiguous nucleotides, or about 30 contiguous nucleotides, preferably at least about 40 nucleotides, or at least about 50 nucleotides, of the sequence in SEQ ID NO:27, preferably excluding the nucleotide sequences determined from the above-listed 4 cDNA clones. The invention also includes a polynucleotide comprising a sequence at least 90%, or at least 95%, identical to any portion of at least about 10 contiguous nucleotides, about 20 contiguous nucleotides, about 25 contiguous nucleotides, or about 30 contiguous nucleotides, preferably at least about 40 nucleotides, or at least about 50 nucleotides, of the sequence in SEQ ID NO:29, preferably excluding the nucleotide sequences determined from the above-listed 4 cDNA clones. The invention also includes a polynucleotide comprising a sequence at least 90%, or at least 95%, identical to any portion of at least about 10 contiguous nucleotides, about 20 contiguous nucleotides, about 25 contiguous nucleotides, or about 30 contiguous nucleotides, preferably at least about 40 nucleotides, or at least about 50 nucleotides, of the sequence in SEQ ID NO:37, preferably excluding the nucleotide sequences determined from the above-listed 4 cDNA clones. In this context "about" includes the particularly recited ranges, larger or smaller by several (i.e. 5, 4, 3, 2 or 1) amino acids, at either extreme or at both extremes.

**[0168]** By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five mismatches per each 100 nucleotides of the reference nucleotide sequence encoding the Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. The reference (query) sequence may be the entire nucleotide sequence encoding Neutrokin- $\alpha$  or Neutrokin- $\alpha$ SV, as shown in Figures 1A and 1B (SEQ ID NO:1) and Figures 5A and 5B (SEQ ID NO:18), respectively, or any Neutrokin- $\alpha$  such as, for example, the Neutrokin- $\alpha$  polynucleotides shown as SEQ ID NOs:21, 22, 27, 29, or 37, or any Neutrokin- $\alpha$  or Neutrokin- $\alpha$ SV polynucleotide fragment as described herein.

**[0169]** As a practical matter, whether any particular nucleic acid molecule is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequences shown in Figures 1A and 1B, or the nucleotide sequences shown in Figures 5A and 5B, or to the nucleotides sequence of the deposited cDNA clones, or to any Neutrokin- $\alpha$  polynucleotide such as, for example, the Neutrokin- $\alpha$  polynucleotides shown as SEQ ID NOs:21, 22, 27, 29, or 37, or fragments thereof, can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). Bestfit uses the local homology algorithm of Smith and Waterman to find the best segment of homology between two sequences (*Advances in Applied Mathematics* 2:482-489 (1981)). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present

invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

[0170] In a specific embodiment, the identity between a reference (query) sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, is determined using the FASTDB computer program based on the algorithm of Brutlag and colleagues (*Comp. App. Biosci.* 6:237-245 (1990)). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter. According to this embodiment, if the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction is made to the results to take into consideration the fact that the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. A determination of whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of this embodiment. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score. For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of

the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are made for the purposes of this embodiment.

**[0171]** The present application is directed to nucleic acid molecules at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences (i.e., polynucleotides) disclosed herein (e.g., those disclosed in Figures 1A and 1B (SEQ ID NO:1) or to the nucleic acid sequence of the deposited cDNAs), irrespective of whether they encode a polypeptide having Neutrokin-alpha and/or Neutrokin-alphaSV functional activity (e.g., biological activity). In addition, the present application is also directed to nucleic acid molecules at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figures 5A and 5B (SEQ ID NO:18) or to the nucleic acid sequence of the deposited cDNA, irrespective of whether they encode a polypeptide having Neutrokin-alphaSV activity. Moreover, the present application is also directed to nucleic acid molecules at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, 99% identical to the nucleic acid sequence shown in SEQ ID NOs:21, 22, 27, 29, or 37, irrespective of whether they encode a polypeptide having Neutrokin-alpha activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having Neutrokin-alpha and/or Neutrokin-alphaSV activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having Neutrokin-alpha and/or Neutrokin-alphaSV activity include, inter alia, (1) isolating the Neutrokin-alpha and/or Neutrokin-alphaSV gene or allelic variants thereof in a cDNA library; (2) *in situ* hybridization (e.g., "FISH") to metaphase chromosomal spreads to



provide precise chromosomal location of the Neutrokin-alpha and/or Neutrokin-alphaSV gene, as described in Verma et al., *Human Chromosomes: A Manual of Basic Techniques*, Pergamon Press, New York (1988); and Northern Blot analysis for detecting Neutrokin-alpha and/or Neutrokin-alphaSV mRNA expression in specific tissues.

**[0172]** Preferred, however, are nucleic acid molecules having sequences at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences disclosed herein (e.g., the nucleotide sequence shown in Figures 1A and 1B (SEQ ID NO:1) and the nucleic acid sequence of the deposited cDNAs, or fragments thereof), which do, in fact, encode a polypeptide having Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide functional activity (e.g., biological activity). Also preferred are nucleic acid molecules having sequences at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figures 5A and 5B (SEQ ID NO:18) or to the nucleic acid sequence of the deposited cDNA which do, in fact, encode a polypeptide having Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide functional activity (e.g., biological activity). Also preferred are nucleic acid molecules having sequences at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown SEQ ID NOs:21, 22, 27, 29, or 37, which do, in fact, encode a polypeptide having Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide functional activity (e.g., biological activity).

**[0173]** By "a polypeptide having Neutrokin-alpha polypeptide functional activity" (e.g., biological activity) and "a polypeptide having Neutrokin-alphaSV polypeptide functional activity" (e.g., biological activity) are intended polypeptides exhibiting activity similar, but not necessarily identical, to an activity of the extracellular domain or the full-length Neutrokin-alpha or Neutrokin-alphaSV polypeptides of the invention, as measured in a particular functional assay (e.g., immunological or biological assay). For example, Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide functional activity can be measured by the ability of a polypeptide sequence described herein to form multimers (e.g., homodimers and homotrimers) with the complete Neutrokin-alpha and/or Neutrokin-alphaSV or extracellular domain of Neutrokin-alpha and/or Neutrokin-alphaSV, and to bind a Neutrokin-alpha and/or Neutrokin-alphaSV ligand. Additionally, Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide functional

activity can be measured by the ability of a polypeptide sequence described herein to form heteromultimers with APRIL (e.g., SEQ ID NO:20 and SEQ ID NO:47) or APRIL fragments or variants, especially the extracellular soluble domain of APRIL (e.g., amino acids 105-250 of SEQ ID NO:47). Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide functional activity can also be measured by determining the ability of a polypeptide of the invention to induce lymphocyte (e.g., B cell) proliferation, differentiation or activation and/or to extend B cell survival. These functional assays can be routinely performed using techniques described herein (e.g., see Example 6) and otherwise known in the art. Additionally, Neutrokine-alpha or Neutrokine-alphaSV polypeptides of the present invention modulate cell proliferation, cytotoxicity, cell survival and cell death. An *in vitro* cell proliferation, cytotoxicity, cell survival, and cell death assay for measuring the effect of a protein on certain cells can be performed by using reagents well known and commonly available in the art for detecting cell replication and/or death. For instance, numerous such assays for TNF-related protein activities are described in the various references in this disclosure. Briefly, an example of such an assay involves collecting human or animal (e.g., mouse) cells and mixing with (1) transfected host cell-supernatant containing Neutrokine-alpha protein (or a candidate polypeptide) or (2) nontransfected host cell-supernatant control, and measuring the effect on cell numbers or viability after incubation of certain period of time. Such cell proliferation and/or survival modulation activities as can be measured in this type of assay are useful for treating tumor, tumor metastasis, infections, autoimmune diseases, inflammation and other immune-related diseases.

**[0174]** Neutrokine-alpha modulates cell proliferation and differentiation in a dose-dependent manner in the above-described assay. Accordingly, it is preferred that "a polypeptide having Neutrokine-alpha polypeptide functional activity" (e.g., biological activity) includes polypeptides that also exhibit any of the same cell modulatory (particularly immunomodulatory) activities in the above-described assays in a dose-dependent manner. Although the degree of dose-dependent activity need not be identical to that of the Neutrokine-alpha polypeptides, preferably, "a polypeptide having Neutrokine-alpha polypeptide functional activity" will exhibit substantially similar dose-dependence in a given activity as compared to the Neutrokine-alpha polypeptides (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold

less and, preferably, not more than about tenfold less activity relative to the reference Neutrokin- $\alpha$  polypeptides).

[0175] In certain preferred embodiments, "a polypeptide having Neutrokin- $\alpha$  polypeptide functional activity" (e.g., biological activity) and "a polypeptide having Neutrokin- $\alpha$ SV polypeptide functional activity" (e.g., biological activity) includes polypeptides that also exhibit any of the same B cell (or other cell type) modulatory (particularly immunomodulatory) activities described in Figures 8A, 8B, 8C, 9A, 9B, 10A, 10B, 10C, 10D, 10E, 10F, 11A, 11B, 11C, 11D, 11E, and 11F and in Example 6.

[0176] Like other members of TNF family, Neutrokin- $\alpha$  exhibits activity on leukocytes including, for example, monocytes, lymphocytes (e.g., B cells) and neutrophils. For this reason Neutrokin- $\alpha$  is active in directing the proliferation, differentiation and migration of these cell types. Such activity is useful for immune enhancement or suppression, myeloprotection, stem cell mobilization, acute and chronic inflammatory control and treatment of leukemia. Assays for measuring such activity are known in the art. For example, see Peters et al., *Immun. Today* 17:273 (1996); Young et al., *J. Exp. Med.* 182:1111 (1995); Caux et al., *Nature* 390:258 (1992); and Santiago-Schwarz et al., *Adv. Exp. Med. Biol.* 378:7 (1995).

[0177] Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence contained in cDNA clone deposited in ATCC accession no. 97768, or the nucleic acid sequence shown in Figures 1A and 1B (SEQ ID NO:1), or fragments thereof, will encode a polypeptide "having Neutrokin- $\alpha$  polypeptide functional activity" (e.g., biological activity). One of ordinary skill in the art will also immediately recognize that a large number of the nucleic acid molecules having a sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence contained in cDNA clone deposited in ATCC accession no. 203518 or the nucleic acid sequence shown in Figures 5A and 5B (SEQ ID NO:18) will encode a polypeptide "having Neutrokin- $\alpha$ SV polypeptide functional activity" (e.g., biological activity). In fact, since degenerate variants of these nucleotide sequences all encode the same polypeptide, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such

nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid), as further described below.

[0178] Similarly, polynucleotides encoding polypeptides which contain all or some portion of the region V-142 through K-160 of SEQ ID NO:2 are likely to be valuable diagnostic and therapeutic polynucleotides with regard to detecting and/or altering expression of either Neutrokin- $\alpha$  or Neutrokin- $\alpha$ SV polynucleotides. In addition, polynucleotides which span the junction of amino acid residues T-141 and G-142 of the Neutrokin- $\alpha$ SV polypeptide shown in SEQ ID NO:19 (in between which the V-142 through K-160 amino acid sequence of Neutrokin- $\alpha$  is apparently inserted), are also likely to be useful both diagnostically and therapeutically. Such T-141/G-142 spanning polynucleotides will exhibit a much higher likelihood of hybridization with Neutrokin- $\alpha$ SV polynucleotides than with Neutrokin- $\alpha$  polynucleotides. A partial, non-limiting, non-exclusive list of such Neutrokin- $\alpha$ SV polypeptides which are encoded by polynucleotides of the invention includes polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the following: G-121 through E-163; E-122 through E-163; G-123 through E-163; N-124 through E-163; S-125 through E-163; S-126 through E-163; Q-127 through E-163; N-128 through E-163; S-129 through E-163; R-130 through E-163; N-131 through E-163; K-132 through E-163; R-133 through E-163; A-134 through E-163; V-135 through E-163; Q-136 through E-163; G-137 through E-163; P-138 through E-163; E-139 through E-163; E-140 through E-163; T-141 through E-163; G-142 through E-163; S-143 through E-163; Y-144 through E-163; T-145 through E-163; F-146 through E-163; V-147 through E-163; P-148 through E-163; W-149 through E-163; L-150 through E-163; L-151 through E-163; S-152 through E-163; F-153 through E-163; K-154 through E-163; R-155 through E-163; G-156 through E-163; S-157 through E-163; A-158 through E-163; L-159 through E-163; E-160 through E-163; E-161 through E-163; K-162 through E-163; G-121 through K-162; G-121 through E-161; G-121 through E-160; G-121 through L-159; G-121 through A-158; G-121 through S-157; G-121 through G-156; G-121 through R-155; G-121 through K-154; G-121 through F-153; G-121 through S-152; G-121 through L-151; G-121 through L-150; G-121 through

W-149; G-121 through P-148; G-121 through V-147; G-121 through F-146; G-121 through T-145; G-121 through Y-144; G-121 through S-143; G-121 through G-142; G-121 through T-141; G-121 through E-140; G-121 through E-139; G-121 through P-138; G-121 through G-137; G-121 through Q-136; G-121 through V-135; G-121 through A-134; G-121 through R-133; G-121 through K-132; G-121 through N-131; G-121 through R-130; G-121 through S-129; G-121 through N-128; G-121 through Q-127; G-121 through S-126; G-121 through S-125; G-121 through N-124; G-121 through G-123; and G-121 through E-122 of SEQ ID NO:19. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

### ***Vectors and Host Cells***

**[0179]** The present invention also relates to vectors which include the isolated DNA molecules of the present invention, host cells which are genetically engineered with the recombinant vectors, or which are otherwise engineered to produce the polypeptides of the invention, and the production of Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides, or fragments thereof, by recombinant or synthetic techniques.

**[0180]** In one embodiment, the polynucleotides of the invention are joined to a vector (e.g., a cloning or expression vector). The vector may be, for example, a phage, plasmid, viral or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells. The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Introduction of the vector construct into the host cell can be effected by techniques known in the art which include, but are not limited to, calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., *Basic Methods In Molecular Biology* (1986).

**[0181]** Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as

3-phosphoglycerate kinase (PGK), a-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, for example, stabilization or simplified purification of expressed recombinant product.

[0182] In one embodiment, the DNA of the invention is operatively associated with an appropriate heterologous regulatory element (e.g., promoter or enhancer), such as, the phage lambda PL promoter, the *E. coli lac*, *trp*, *phoA*, and *tac* promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan.

[0183] As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells (e.g., *Saccharomyces cerevisiae* or *Pichia pastoris* (ATCC Accession No. 201178)); insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, 293 and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

[0184] The host cell can be a higher eukaryotic cell, such as a mammalian cell (e.g., a human derived cell), or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. The host strain may be chosen which modulates the expression of the inserted gene sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus expression of the genetically engineered polypeptide may be controlled. Furthermore, different host cells have characteristics and specific mechanisms for the translational and post-translational processing and modification (e.g., phosphorylation, cleavage) of proteins. Appropriate cell lines can be chosen to ensure the desired modifications and processing of the foreign protein

expressed. Selection of appropriate vectors and promoters for expression in a host cell is a well-known procedure and the requisite techniques for expression vector construction, introduction of the vector into the host and expression in the host are routine skills in the art.

[0185] Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium*, and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice. As a representative, but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well-known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. Among vectors preferred for use in bacteria include pHE4-5 (ATCC Accession No. 209311; and variations thereof), pQE70, pQE60 and pQE-9, available from QIAGEN, Inc., *supra*; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlsbad, CA). Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL (available from Pharmacia). Other suitable vectors will be readily apparent to the skilled artisan.

[0186] Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g.,

temperature shift or chemical induction) and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

[0187] Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well known to those skilled in the art.

[0188] In one embodiment, the yeast *Pichia pastoris* is used to express Neutrokin- $\alpha$  protein in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A main step in the methanol metabolism pathway is the oxidation of methanol to formaldehyde using  $O_2$ . This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for  $O_2$ . Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (*AOX1*) is highly active. In the presence of methanol, alcohol oxidase produced from the *AOX1* gene comprises up to approximately 30% of the total soluble protein in *Pichia pastoris*. See, Ellis, S.B., *et al.*, *Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J., *et al.*, *Yeast* 5:167-77 (1989); Tschopp, J.F., *et al.*, *Nucl. Acids Res.* 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a Neutrokin- $\alpha$  or Neutrokin- $\alpha$ SV polynucleotide of the present invention, under the transcriptional regulation of all or part of the *AOX1* regulatory sequence is expressed at exceptionally high levels in *Pichia* yeast grown in the presence of methanol.

[0189] In one example, the plasmid vector pPIC9K is used to express DNA encoding a Neutrokin- $\alpha$  or Neutrokin- $\alpha$ SV polypeptide of the invention, as set forth herein, in a *Pichea* yeast system essentially as described in "*Pichia* Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a Neutrokin- $\alpha$  or Neutrokin- $\alpha$ SV protein of the invention by virtue of the strong *AOX1* promoter linked to the *Pichia pastoris* alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.



[0190] Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

[0191] In one embodiment, high-level expression of a heterologous coding sequence, such as, for example, a Neutrokin-alpha or Neutrokin-alphaSV polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

[0192] Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are *cis*-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

[0193] Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman (*Cell* 23:175 (1981)), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

[0194] In a specific embodiment, constructs designed to express a portion of the extracellular domain of the Neutrokin-alpha (e.g., amino acid residues Ala-134 through Leu-285) are preferred. One of skill in the art would be able to use the polynucleotide and polypeptide sequences provided as SEQ ID NO:1 and SEQ ID NO:2, respectively, or SEQ

ID NO:18 and SEQ ID NO:19, respectively, to design polynucleotide primers to generate such an expression construct.

[0195] In another embodiment, constructs designed to express the entire predicted extracellular domain of the Neutrokin- $\alpha$  (i.e., amino acid residues Gln-73 through Leu-285) are preferred. One of skill in the art would be able to use the polynucleotide and polypeptide sequences provided as SEQ ID NO:1 and SEQ ID NO:2, respectively, or SEQ ID NO:18 and SEQ ID NO:19, respectively, to design polynucleotide primers to generate such an expression construct.

[0196] In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., Neutrokin- $\alpha$  coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with Neutrokin- $\alpha$  polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous Neutrokin- $\alpha$  polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous Neutrokin- $\alpha$  polynucleotide sequences via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

[0197] The host cells described *infra* can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, cell-free translation systems can also be employed to produce the polypeptides of the invention using RNAs derived from the DNA constructs of the present invention.

[0198] The polypeptide of the invention may be expressed or synthesized in a modified form, such as a fusion protein (comprising the polypeptide joined via a peptide bond to a heterologous protein sequence (of a different protein)), and may include not only secretion signals, but also additional heterologous functional regions. Such a fusion protein can be made by ligating polynucleotides of the invention and the desired nucleic

acid sequence encoding the desired amino acid sequence to each other, by methods known in the art, in the proper reading frame, and expressing the fusion protein product by methods known in the art. Alternatively, such a fusion protein can be made by protein synthetic techniques, e.g., by use of a peptide synthesizer. Thus, for instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art.

**[0199]** In one embodiment, polynucleotides encoding Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides of the invention may be fused to signal sequences which will direct the localization of a protein of the invention to particular compartments of a prokaryotic or eukaryotic cell and/or direct the secretion of a protein of the invention from a prokaryotic or eukaryotic cell. For example, in *E. coli*, one may wish to direct the expression of the protein to the periplasmic space. Examples of signal sequences or proteins (or fragments thereof) to which the polypeptides of the invention may be fused in order to direct the expression of the polypeptide to the periplasmic space of bacteria include, but are not limited to, the *pelB* signal sequence, the maltose binding protein (MBP) signal sequence, MBP, the *ompA* signal sequence, the signal sequence of the periplasmic *E. coli* heat-labile enterotoxin B-subunit, and the signal sequence of alkaline phosphatase. Several vectors are commercially available for the construction of fusion proteins which will direct the localization of a protein, such as the pMAL series of vectors (particularly the pMAL-p series) available from New England Biolabs. In a specific embodiment, polynucleotides encoding Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides of the invention may be fused to the *pelB* peptidase signal sequence to increase the efficiency of expression and purification of such polypeptides in Gram-negative bacteria. *See*, U.S. Patent Nos. 5,576,195 and 5,846,818, the contents of which are herein incorporated by reference in their entireties.

**[0200]** Examples of signal peptides that may be fused to a polypeptide of the invention in order to direct its secretion in mammalian cells include, but are not limited to, the

MPIF-1 signal sequence (amino acids 1-21 of GenBank Accession number AAB51134), the stanniocalcin signal sequence (MLQNSAVLLLLVISASA, SEQ ID NO:45), and a consensus signal sequence (MPTWAWWLFLVLLLALWAPARG, SEQ ID NO:46). A suitable signal sequence that may be used in conjunction with baculoviral expression systems is the gp67 signal sequence, (amino acids 1-19 of GenBank Accession Number AAA72759).

[0201] A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to stabilize and purify proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5 has been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett et al., *J. Molecular Recognition* 8:52-58 (1995) and K. Johanson et al., *J. Biol. Chem.* 270:9459-9471 (1995).

[0202] Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

[0203] Polypeptides of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, *Proteins: Structures and Molecular Principles*, W.H. Freeman & Co., N.Y., and Hunkapiller, M., et al., 1984, *Nature* 310:105-111). For example, a peptide corresponding to a fragment of the complete Neutrokin- $\alpha$  or

Neutrokin- $\alpha$ SV polypeptides of the invention can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the Neutrokin- $\alpha$  or Neutrokin- $\alpha$ SV polynucleotide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid,  $\alpha$ -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid,  $\gamma$ -Abu,  $\epsilon$ -Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine,  $\beta$ -alanine, fluoro-amino acids, designer amino acids such as  $\beta$ -methyl amino acids,  $\alpha$ -methyl amino acids,  $N$ -methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

[0204] The invention encompasses Neutrokin- $\alpha$  or Neutrokin- $\alpha$ SV polypeptides which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease,  $\text{NaBH}_4$ , acetylation, formylation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin, etc.

[0205] Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, radioisotopic or affinity label to allow for detection and isolation of the protein.

[0206] Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase, glucose oxidase or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include biotin, umbelliferone, fluorescein,

fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include a radioactive metal ion, e.g., alpha-emitters such as, for example,  $^{213}\text{Bi}$ , or other radioisotopes such as, for example, iodine ( $^{131}\text{I}$ ,  $^{125}\text{I}$ ,  $^{123}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{115\text{m}}\text{In}$ ,  $^{113\text{m}}\text{In}$ ,  $^{112}\text{In}$ ,  $^{111}\text{In}$ ), and technetium ( $^{99}\text{Tc}$ ,  $^{99\text{m}}\text{Tc}$ ), thallium ( $^{201}\text{Tl}$ ), gallium ( $^{68}\text{Ga}$ ,  $^{67}\text{Ga}$ ), palladium ( $^{103}\text{Pd}$ ), molybdenum ( $^{99}\text{Mo}$ ), xenon ( $^{133}\text{Xe}$ ), fluorine ( $^{18}\text{F}$ ),  $^{153}\text{Sm}$ ,  $^{177}\text{Lu}$ ,  $^{159}\text{Gd}$ ,  $^{149}\text{Pm}$ ,  $^{140}\text{La}$ ,  $^{175}\text{Yb}$ ,  $^{166}\text{Ho}$ ,  $^{90}\text{Y}$ ,  $^{47}\text{Sc}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$ ,  $^{142}\text{Pr}$ ,  $^{105}\text{Rh}$ ,  $^{97}\text{Ru}$ ,  $^{68}\text{Ge}$ ,  $^{57}\text{Co}$ ,  $^{65}\text{Zn}$ ,  $^{85}\text{Sr}$ ,  $^{32}\text{P}$ ,  $^{153}\text{Gd}$ ,  $^{169}\text{Yb}$ ,  $^{51}\text{Cr}$ ,  $^{54}\text{Mn}$ ,  $^{75}\text{Se}$ ,  $^{113}\text{Sn}$ , and  $^{117}\text{In}$ .

[0207] In specific embodiments, Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides of the invention may be labeled with Europium. For example, Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides of the invention may be labelled with Europium using the DELFIA Eu-labeling kit (catalog# 1244-302, Perkin Elmer Life Sciences, Boston, MA) following manufacturer's instructions.

[0208] In specific embodiments, Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides of the invention are attached to macrocyclic chelators useful for conjugating radiometal ions, including but not limited to,  $^{111}\text{In}$ ,  $^{177}\text{Lu}$ ,  $^{90}\text{Y}$ ,  $^{166}\text{Ho}$ , and  $^{153}\text{Sm}$ , to polypeptides. In a preferred embodiment, the radiometal ion associated with the macrocyclic chelators attached to Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides of the invention is  $^{111}\text{In}$ . In another preferred embodiment, the radiometal ion associated with the macrocyclic chelator attached to Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides of the invention is  $^{90}\text{Y}$ . In specific embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA). In other specific embodiments, the DOTA is attached to the Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide of the invention via a linker molecule. Examples of linker molecules useful for conjugating DOTA to a polypeptide are commonly known in the art - see, for example, DeNardo et al., Clin Cancer Res. 4(10):2483-90, 1998; Peterson et al., Bioconjug. Chem. 10(4):553-7, 1999; and Zimmerman et al, Nucl. Med. Biol. 26(8):943-50, 1999 which are hereby incorporated by reference in their entirety. In addition, U.S. Patents 5,652,361 and 5,756,065, which disclose chelating agents that may be conjugated to antibodies, and methods for making and using them, are hereby

incorporated by reference in their entireties. Though U.S. Patents 5,652,361 and 5,756,065 focus on conjugating chelating agents to antibodies, one skilled in the art could readily adapt the method disclosed therein in order to conjugate chelating agents to other polypeptides.

[0209] In one embodiment, Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides of the invention may be labeled with biotin. In other related embodiments, biotinylated Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides of the invention may be used, for example, as an imaging agent or as a means of identifying one or more Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV receptor(s) or other coreceptor or coligand molecules.

[0210] Also provided by the invention are chemically modified derivatives of Neutrokin- $\alpha$  or Neutrokin- $\alpha$ SV which may provide additional advantages such as increased solubility, stability and in vivo or in vitro circulating time of the polypeptide, or decreased immunogenicity (see U. S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

[0211] The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500,

20,000, 25,000, 30,000, 35,000, 40,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

**[0212]** As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo *et al.*, *Appl. Biochem. Biotechnol.* 56:59-72 (1996); Vorobjev *et al.*, *Nucleosides Nucleotides* 18:2745-2750 (1999); and Caliceti *et al.*, *Bioconjug. Chem.* 10:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

**[0213]** The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik *et al.*, *Exp. Hematol.* 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include, for example, lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues, glutamic acid residues, and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

**[0214]** As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to a proteins via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

**[0215]** One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene



glycol molecules to protein (or peptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

[0216] As indicated above, pegylation of the proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the protein either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992); Francis *et al.*, *Intern. J. of Hematol.* 68:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

[0217] One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride ( $\text{ClSO}_2\text{CH}_2\text{CF}_3$ ). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoroethane sulphonyl group.

[0218] Polyethylene glycol can also be attached to proteins using a number of different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-

nitrophenolcarbonate, and various MPEG-succinate derivatives. A number additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated protein products produced using the reaction chemistries set out herein are included within the scope of the invention.

[0219] The number of polyethylene glycol moieties attached to each protein of the invention (*i.e.*, the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992).

[0220] The Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides can be recovered and purified by known methods which include, but are not limited to, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

### ***Neutrokin- $\alpha$ Polypeptides***

[0221] The Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides of the invention may be in monomers or multimers (*i.e.*, dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides of the invention, their preparation, and compositions (preferably, pharmaceutical compositions) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

[0222] In a nonexclusive embodiment, the multimers of the invention comprise at least one Neutrokin- $\alpha$  – human serum albumin fusion protein as described herein. In

another nonexclusive embodiment, the multimers of the invention are trimeric and comprise one, two or three Neutrokin-alpha – human serum albumin fusion proteins as described herein.

**[0223]** Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides of the invention (including Neutrokin-alpha and/or Neutrokin-alphaSV fragments, variants, and fusion proteins, as described herein). These homomers may contain Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the invention is a multimer containing Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (e.g., containing Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides having identical or different amino acid sequences) or a homotrimer (e.g., containing Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides having identical or different amino acid sequences). In a preferred embodiment, the multimer of the invention is a homotrimer. In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

**[0224]** As used herein, the term heteromer refers to a multimer containing heterologous polypeptides (i.e., polypeptides of a different protein) in addition to the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer. In highly preferred embodiments, the heteromeric multimer of the invention is a heterotrimer comprising both Neutrokin alpha-polypeptides and APRIL polypeptides (e.g., SEQ ID NO:20 or SEQ ID NO:47; PCT International Publication Number WO97/33902; GenBank Accession No. AF046888 (nucleotide) and AAC6132 (protein); J. Exp. Med. 188(6):1185-1190). In other highly preferred embodiments, the heteromeric multimer of the invention is a heterotrimer consisting of one Neutrokin alpha-polypeptide and two

APRIL polypeptides. In other highly preferred embodiments, the heteromeric multimer of the invention is a heterotrimer consisting of two Neutrokin alpha-polypeptides and one APRIL polypeptide.

[0225] In a further nonexclusive embodiment, the heteromers of the invention comprise CD40 ligand polypeptide sequence(s), or biologically active fragment(s) or variant(s) thereof.

[0226] Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence (e.g., that recited in SEQ ID NO:2 or SEQ ID NO:19, or contained in the polypeptide encoded by the clones deposited in connection with this application). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a Neutrokin-alpha and/or Neutrokin-alphaSV fusion protein. In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in a Neutrokin-alpha-Fc and/or Neutrokin-alphaSV-Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another TNF family ligand/receptor member that is capable of forming covalently associated multimers, such

as for example, osteoprotegerin (see, e.g., International Publication No. WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from CD40L, or a soluble fragment thereof. In another embodiment, two or more Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$  polypeptides of the invention are joined through synthetic linkers (e.g., peptide, carbohydrate or soluble polymer linkers). Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides separated by peptide linkers may be produced using conventional recombinant DNA technology.

**[0227]** Another method for preparing multimer Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides of the invention involves use of Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper or isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., *Science* 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers or isoleucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV proteins are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a soluble Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide fused to a peptide that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV is recovered from the culture supernatant using techniques known in the art.

**[0228]** Certain members of the TNF family of proteins are believed to exist in trimeric form (Beutler and Hufel, *Science* 264:667, 1994; Banner et al., *Cell* 73:431, 1993). Thus, trimeric Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (*FEBS Letters* 344:191, (1994)) and in U.S.

patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric Neutrokin-alpha and/or Neutrokin-alphaSV.

**[0229]** In another example, proteins of the invention are associated by interactions between the Flag® polypeptide sequence contained in Flag®-Neutrokin alpha or Flag®-Neutrokin-alphaSV fusion proteins of the invention. In a further embodiment, proteins of the invention are associated by interactions between the heterologous polypeptide sequence contained in Flag®-Neutrokin-alpha or Flag®-Neutrokin-alphaSV fusion proteins of the invention and anti-Flag® antibody.

**[0230]** The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely modified by the addition of cysteine or biotin to the C terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

**[0231]** Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a

polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

**[0232]** In one embodiment, the invention provides an isolated Neutrokin- $\alpha$  polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC No. 97768, or the amino acid sequence in Figures 1A and 1B (SEQ ID NO:2), or a polypeptide comprising a portion (i.e., a fragment) of the above polypeptides. In another embodiment, the invention provides an isolated Neutrokin- $\alpha$ SV polypeptide having the amino acid encoded by the cDNA clone contained in ATCC No. 203518, or the amino acid sequence in Figures 5A and 5B (SEQ ID NO:19), or a polypeptide comprising a portion (i.e., fragment) of the above polypeptides.

**[0233]** Polypeptide fragments of the present invention include polypeptides comprising or alternatively, consisting of, an amino acid sequence contained in SEQ ID NO:2, encoded by the cDNA contained in the plasmid having ATCC accession number 97768, or encoded by nucleic acids which hybridize (e.g., under stringent hybridization conditions) to the nucleotide sequence contained in the deposited clone, or the complementary strand of the nucleotide sequence shown in Figures 1A-B (SEQ ID NO:1).

**[0234]** Additionally, polypeptide fragments of the present invention include polypeptides comprising or alternatively, consisting of, an amino acid sequence contained in SEQ ID NO:19, encoded by the cDNA contained in the plasmid having ATCC accession number 203518, or encoded by nucleic acids which hybridize (e.g., under stringent hybridization conditions) to the nucleotide sequence contained in the deposited clone, or the complementary strand of the nucleotide sequence shown in Figures 5A-B (SEQ ID NO:18).

**[0235]** Additionally, polypeptide fragments of the present invention include polypeptides comprising or alternatively, consisting of, an amino acid sequence encoded by nucleic acids which hybridize (e.g., under hybridization conditions described herein) to the complementary strand of the nucleotide sequence shown in SEQ ID NO:21.

**[0236]** Polypeptide fragments of the present invention also include polypeptides comprising or alternatively, consisting of, an amino acid sequence contained in SEQ ID NO:23, or encoded by nucleic acids which hybridize (e.g., under hybridization conditions described herein) to the complementary strand of the nucleotide sequence shown in SEQ ID NO:22.

**[0237]** In addition, polypeptide fragments of the present invention include polypeptides comprising or alternatively, consisting of, an amino acid sequence contained in SEQ ID NO:28, or encoded by nucleic acids which hybridize (e.g., under hybridization conditions described herein) to the complementary strand of the nucleotide sequence shown in SEQ ID NO:27.

**[0238]** Additionally, polypeptide fragments of the present invention include polypeptides comprising or alternatively, consisting of, an amino acid sequence contained in SEQ ID NO:30, or encoded by nucleic acids which hybridize (e.g., under hybridization conditions described herein) to the complementary strand of the nucleotide sequence shown in SEQ ID NO:29.

**[0239]** Additionally, polypeptide fragments of the present invention include polypeptides comprising or alternatively, consisting of, an amino acid sequence contained in SEQ ID NO:38, or encoded by nucleic acids which hybridize (e.g., under hybridization conditions described herein) to the complementary strand of the nucleotide sequence shown in SEQ ID NO:37.

**[0240]** Additionally, polypeptide fragments of the present invention include polypeptides comprising or alternatively, consisting of, an amino acid sequence contained in SEQ ID NO:39, or encoded by nucleic acids which hybridize (e.g., under hybridization conditions described herein) to the complementary strand of a nucleotide sequence encoding the polypeptide of SEQ ID NO:39.

**[0241]** Additionally, polypeptide fragments of the present invention include polypeptides comprising or alternatively, consisting of, an amino acid sequence contained in SEQ ID NO:40, or encoded by nucleic acids which hybridize (e.g., under hybridization



conditions described herein) to the complementary strand of a nucleotide sequence encoding the polypeptide of SEQ ID NO:40.

[0242] Additionally, polypeptide fragments of the present invention include polypeptides comprising or alternatively, consisting of, an amino acid sequence contained in SEQ ID NO:41, or encoded by nucleic acids which hybridize (e.g., under hybridization conditions described herein) to the complementary strand of a nucleotide sequence encoding the polypeptide of SEQ ID NO:41.

[0243] Additionally, polypeptide fragments of the present invention include polypeptides comprising or alternatively, consisting of, an amino acid sequence contained in SEQ ID NO:42, or encoded by nucleic acids which hybridize (e.g., under hybridization conditions described herein) to the complementary strand of a nucleotide sequence encoding the polypeptide of SEQ ID NO:42.

[0244] Additionally, polypeptide fragments of the present invention include polypeptides comprising or alternatively, consisting of, an amino acid sequence contained in SEQ ID NO:43, or encoded by nucleic acids which hybridize (e.g., under hybridization conditions described herein) to the complementary strand of a nucleotide sequence encoding the polypeptide of SEQ ID NO:43.

[0245] Additionally, polypeptide fragments of the present invention include polypeptides comprising or alternatively, consisting of, an amino acid sequence contained in SEQ ID NO:44, or encoded by nucleic acids which hybridize (e.g., under hybridization conditions described herein) to the complementary strand of a nucleotide sequence encoding the polypeptide of SEQ ID NO:44.

[0246] Polypeptide fragments of the present invention include polypeptides comprising or alternatively, consisting of, an amino acid sequence contained in SEQ ID NO:2, encoded by the cDNA contained in the deposited clone, or encoded by nucleic acids which hybridize (e.g., under stringent hybridization conditions) to the nucleotide sequence contained in the deposited clone, or shown in Figures 1A and 1B (SEQ ID NO:1) or the complementary strand thereto. Protein fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments that comprise or alternatively, consist of from about amino acid residues: 1 to 50, 51 to 100, 101 to 150, 151 to 200, 201

to 250, and/or 251 to 285 of SEQ ID NO:2. Moreover, polypeptide fragments can be at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 175 or 200 amino acids in length.

**[0247]** In specific embodiments, polypeptide fragments of the invention comprise, or alternatively consist of, amino acid residues: 1-46, 31-44, 47-72, 73-285, 73-83, 94-102, 148-152, 166-181, 185-209, 210-221, 226-237, 244-249, 253-265, and/or 277-284, as depicted in Figures 1A and 1B (SEQ ID NO:2). Polynucleotides encoding these polypeptides are also encompassed by the invention.

**[0248]** It will be recognized by one of ordinary skill in the art that mutations targeted to regions of a Neutrokin- $\alpha$  polypeptide of the invention which encompass the nineteen amino acid residue insertion which is not found in the Neutrokin- $\alpha$ SV polypeptide sequence (i.e., amino acid residues Val-142 through Lys-160 of the sequence presented in Figures 1A and 1B and in SEQ ID NO:2) may affect the observed biological activities of the Neutrokin- $\alpha$  polypeptide. More specifically, a partial, non-limiting and non-exclusive list of such residues of the Neutrokin- $\alpha$  polypeptide sequence which may be targeted for mutation includes the following amino acid residues of the Neutrokin- $\alpha$  polypeptide sequence as shown in SEQ ID NO:2: V-142; T-143; Q-144; D-145; C-146; L-147; Q-148; L-149; I-150; A-151; D-152; S-153; E-154; T-155; P-156; T-157; I-158; Q-159; and K-160. Polynucleotides encoding Neutrokin- $\alpha$  polypeptides which have one or more mutations in the region from V-142 through K-160 of SEQ ID NO:2 are contemplated. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

**[0249]** Polypeptide fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments that comprise or alternatively, consist of from about amino acid residues: 1 to 15, 16-30, 31-46, 47-55, 56-72, 73-104, 105-163, 163-188, 186-210 and 210-284 of the amino acid sequence disclosed in SEQ ID NO:2. Additional representative examples of polypeptide fragments of the invention, include, for example, fragments that comprise or alternatively, consist of from about amino acid residues: 1 to 143, 1-150, 47-143, 47-150, 73-143, 73-150, 100-150, 140-145, 142-148, 140-150, 140-200, 140-225, and 140-266 of the amino acid sequence disclosed in SEQ ID NO:19.

Moreover, polypeptide fragments can be at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 175 or 200 amino acids in length. In this context, "about" means the particularly recited ranges and ranges larger or smaller by several, a few, 5, 4, 3, 2 or 1 amino acid residues at either or both the amino- and carboxy-termini. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

**[0250]** Additional preferred embodiments encompass polypeptide fragments comprising, or alternatively consisting of, the predicted intracellular domain of Neutrokin- $\alpha$  (amino acid residues 1-46 of SEQ ID NO:2), the predicted transmembrane domain of Neutrokin- $\alpha$  (amino acid residues 47-72 of SEQ ID NO:2), the predicted extracellular domain of Neutrokin- $\alpha$  (amino acid residues 73-285 of SEQ ID NO:2), the predicted TNF conserved domain of Neutrokin- $\alpha$  (amino acids 191 to 284 of SEQ ID NO:2), and a polypeptide comprising, or alternatively, consisting of the predicted intracellular domain fused to the predicted extracellular domain of Neutrokin- $\alpha$  (amino acid residues 1-46 fused to amino acid residues 73-285 of SEQ ID NO:2). Polynucleotides encoding these polypeptides are also encompassed by the invention.

**[0251]** Further additional preferred embodiments encompass polypeptide fragments comprising, or alternatively consisting of, the predicted intracellular domain of Neutrokin- $\alpha$ SV (amino acid residues 1-46 of SEQ ID NO:19), the predicted transmembrane domain of Neutrokin- $\alpha$ SV (amino acid residues 47-72 of SEQ ID NO:19), the predicted extracellular domain of Neutrokin- $\alpha$ SV (amino acid residues 73-266 of SEQ ID NO:19), the predicted TNF conserved domain of Neutrokin- $\alpha$ SV (amino acids 172 to 265 of SEQ ID NO:19), and a polypeptide comprising, or alternatively, consisting of the predicted intracellular domain fused to the predicted extracellular domain of Neutrokin- $\alpha$ SV (amino acid residues 1-46 fused to amino acid residues 73-266 of SEQ ID NO:19). Polynucleotides encoding these polypeptides are also encompassed by the invention.

**[0252]** Certain additional embodiments of the invention encompass polypeptide fragments comprising, or alternatively consisting of, the predicted beta-pleated sheet regions identified in Figures 7A-1 and 7A-2. These polypeptide fragments of the invention comprise, or alternatively consist of, amino acid residues Gln-144 to Ala-151, Phe-172 to Lys-173, Ala-177 to Glu-179, Asn-183 to Ile-185, Gly-191 to Lys-204, His-

210 to Val-219, Leu-226 to Pro-237, Asn-242 to Ala-251, Gly-256 to Ile-263 and/or Val-276 to Leu-284 of SEQ ID NO:2. In another, nonexclusive embodiment, these polypeptide fragments of the invention also comprise, or alternatively consist of, amino acid residues Phe-153 to Lys-154, Ala-158 to Glu-160, Asn-164 to Ile-166, Gly-172 to Lys-185, His-191 to Val-200, Leu-207 to Pro-218, Asn-223 to Ala-232, Gly-237 to Ile-244 and/or Val-257 to Leu-265 of SEQ ID NO:19; and amino acid residues Phe-42 to Lys-43, Ala-47 to Glu-49, Asn-53 to Ile-55, Gly-61 to Pro-74, His-80 to Val-89, Leu-96 to Pro-107, Asn-112 to Ala-121, Gly-126 to Ile-133 and/or Asp-146 to Leu-154 of SEQ ID NO:23. In further nonexclusive embodiments, these polypeptide fragments of the invention also comprise, or alternatively consist of, amino acid residues Gln-78 to Ala-85; Phe-106 to Lys-107, Ala-111 to Glu-113, Asn-117 to Ile-119, Gly-125 to Lys-138, His-144 to Val-153, Leu-160 to Pro-171, Asn-176 to Ala-185, Gly-190 to Ile-197 and/or Val-210 to Leu-218 of SEQ ID NO:28; and amino acid residues Gln-78 to Ala-85; Phe-106 to Lys-107, Ala-111 to Glu-113, Asn-117 to Ile-119, Gly-125 to Lys-138, His-144 to Val-153, Leu-160 to Pro-171, Asn-176 to Ala-185, Gly-190 to Ile-197 and/or Val-210 to Leu-218 of SEQ ID NO:30. Polynucleotides encoding these polypeptide fragments are also provided.

[0253] A partial, non-limiting, and exemplary list of polypeptides of the invention which comprise, or alternatively consist of, combinations of amino acid sequences of the invention includes, for example, [Met-1 to Lys-113] fused to [Leu-114 to Thr-141] fused to [Val-142 to Lys-160] fused to [Gly-161 to Gln-198] fused to [Val-199 to Ala-248] fused to [Gly-249 to Leu-285] of SEQ ID NO:2; or [Met-1 to Lys-113] fused to [Val-142 to Lys-160] fused to [Gly-161 to Gln-198] fused to [Val-199 to Ala-248] fused to [Gly-249 to Leu-285] of SEQ ID NO:2; or [Met-1 to Lys-113] fused to [Leu-114 to Thr-141] fused to [Val-142 to Lys-160] fused to [Gly-161 to Gln-198] fused to [Gly-249 to Leu-285] of SEQ ID NO:2. Other combinations may include the polypeptide fragments in an order other than that recited above (e.g., [Leu-114 to Thr-141] fused to [Val-199 to Ala-248] fused to [Gly-249 to Leu-285] fused to [Val-142 to Lys-160] of SEQ ID NO:2). Other combinations may also include heterologous polypeptide fragments as described herein and/or other polypeptides or polypeptide fragments of the present invention (e.g., [Met-1 to Lys-113] fused to [Leu-114 to Thr-141] fused to [Val-142 to Lys-160] fused to [Gly-161 to Gln-198] fused to [Gly-249 to Leu-285] of SEQ ID NO:2 fused to a FLAG

tag; or [Met-1 to Lys-113] of SEQ ID NO:2 fused to [Leu-114 to Thr-141] of SEQ ID NO:2 fused to [Glu-135 to Asn-165] of SEQ ID NO:39 fused to [Val-142 to Lys-160] of SEQ ID NO:2 fused to [Gly-161 to Gln-198] of SEQ ID NO:2 fused to [Val-199 to Ala-248] of SEQ ID NO:2 fused to [Gly-249 to Leu-285] of SEQ ID NO:2). Polynucleotides encoding any of these polypeptides are encompassed by the invention.

**[0254]** An additional partial, non-limiting, and exemplary list of polypeptides of the invention which comprise, or alternatively consist of, combinations of amino acid sequences includes, for example, [Met-1 to Lys-113] fused to [Leu-114 to Thr-141] fused to [Gly-142 to Gln-179] fused to [Val-180 to Ala-229] fused to [Gly-230 to Leu-266] of SEQ ID NO:19; [Met-1 to Lys-113] fused to [Gly-142 to Gln-179] fused to [Val-180 to Ala-229] fused to [Gly-230 to Leu-266] of SEQ ID NO:19; or [Met-1 to Lys-113] fused to [Leu-114 to Thr-141] fused to [Gly-142 to Gln-179] fused to [Gly-230 to Leu-266] of SEQ ID NO:19. Other combinations may include the polypeptide fragments in an order other than that recited above (e.g., [Leu-114 to Thr-141] fused to [Val-180 to Ala-229] fused to [Gly-230 to Leu-266] fused to [Gly-142 to Gln-179] of SEQ ID NO:19). Other combinations may also include heterologous polypeptide fragments as described herein and/or other polypeptides or polypeptide fragments of the present invention (e.g., [Met-1 to Lys-113] fused to [Leu-114 to Thr-141] fused to [Gly-142 to Gln-179] fused to [Gly-230 to Leu-266] of SEQ ID NO:19 fused to a FLAG tag or, [Met-1 to Lys-113] of SEQ ID NO:19 fused to [Leu-114 to Thr-141] of SEQ ID NO:19 fused to [Glu-135 to Asn-165] of SEQ ID NO:39 fused to [Gly-142 to Gln-179] of SEQ ID NO:19 fused to [Val-180 to Ala-229] of SEQ ID NO:19 fused to [Gly-230 to Leu-266] of SEQ ID NO:19). Polynucleotides encoding any of these polypeptides are encompassed by the invention.

**[0255]** A further partial, non-limiting, and exemplary list of polypeptides of the invention which comprise, or alternatively consist of, combinations of amino acid sequences includes, for example, [Met-1 to Lys-106] fused to [Leu-107 to Thr-134] fused to [Ile-166 to Lys-184] fused to [Gly-185 to Gln-222] fused to [Val-223 to Ala-272] fused to [Gly-273 to Leu-309] of SEQ ID NO:39; [Met-1 to Lys-106] fused to [Glu-135 to Asn-165] fused to [Ile-166 to Lys-184] fused to [Gly-185 to Gln-222] fused to [Val-223 to Ala-272] fused to [Gly-273 to Leu-309] of SEQ ID NO:39; or [Met-1 to Lys-106] fused to [Leu-107 to Thr-134] fused to [Glu-135 to Asn-165] fused to [Ile-166 to Lys-184] fused to [Gly-185 to Gln-222] fused to [Gly-273 to Leu-309] of SEQ ID NO:39. Other

combinations may include the polypeptide fragments in an order other than that recited above (e.g., [Met-1 to Lys-106] fused to [Gly-185 to Gln-222] fused to [Ile-166 to Lys-184] fused to [Val-223 to Ala-272] fused to [Leu-107 to Thr-134] fused to [Gly-273 to Leu-309] of SEQ ID NO:39). Other combinations may also include heterologous polypeptide fragments as described herein and/or other polypeptides or polypeptide fragments of the present invention (e.g., [Met-1 to Lys-106] fused to [Glu-135 to Asn-165] fused to [Ile-166 to Lys-184] fused to [Gly-185 to Gln-222] fused to [Val-223 to Ala-272] fused to [Gly-273 to Leu-309] of SEQ ID NO:39 fused to a FLAG tag). Polynucleotides encoding any of these polypeptides are encompassed by the invention.

**[0256]** A further partial, non-limiting, and exemplary list of polypeptides of the invention which comprise, or alternatively consist of, combinations of amino acid sequences includes, for example, [Met-1 to Lys-106] fused to [Leu-107 to Thr-134] fused to [Glu-135 to Asn-165] fused to [Ile-166 to Pro-180] fused to [Ala-181 to Gln-202] fused to [Val-203 to Ala-252] fused to [Gly-253 to Leu-289] of SEQ ID NO:38; [Met-1 to Lys-106] fused to [Leu-107 to Thr-134] fused to [Ile-166 to Pro-180] fused to [Ala-181 to Gln-202] fused to [Val-203 to Ala-252] fused to [Gly-253 to Leu-289] of SEQ ID NO:38; [Met-1 to Lys-106] fused to [Leu-107 to Thr-134] fused to [Glu-135 to Asn-165] fused to [Ala-181 to Gln-202] fused to [Val-203 to Ala-252] fused to [Gly-253 to Leu-289] of SEQ ID NO:38; [Met-1 to Lys-106] fused to [Leu-107 to Thr-134] fused to [Ala-181 to Gln-202] fused to [Val-203 to Ala-252] fused to [Gly-253 to Leu-289] of SEQ ID NO:38; Other combinations may include the polypeptide fragments in an order other than that recited above (e.g., [Met-1 to Lys-106] fused to [Ala-181 to Gln-202] fused to [Ile-166 to Pro-180] fused to [Val-203 to Ala-252] fused to [Leu-107 to Thr-134] fused to [Gly-253 to Leu-289] of SEQ ID NO:38). Other combinations may also include heterologous polypeptide fragments as described herein and/or other polypeptides or polypeptide fragments of the present invention (e.g., [Met-1 to Lys-106] fused to [Glu-135 to Asn-165] fused to [Ile-166 to Pro-180] fused to [Ala-181 to Gln-202] fused to [Val-203 to Ala-252] fused to [Gly-253 to Leu-289] of SEQ ID NO:38 fused to a FLAG tag). Polynucleotides encoding any of these polypeptides are encompassed by the invention.

**[0257]** A further partial, non-limiting, and exemplary list of polypeptides of the invention which comprise, or alternatively consist of, combinations of amino acid sequences includes, for example, [Met-1 to Lys-106] fused to [Leu-107 to Thr-134] fused

to [Glu-135 to Asn-165] fused to [Arg-166 to Gln-203] fused to [Val-204 to Ala-253] fused to [Gly-254 to Leu-290] of SEQ ID NO:40; [Met-1 to Lys-106] fused [Glu-135 to Asn-165] fused to [Arg-166 to Gln-203] fused to [Val-204 to Ala-253] fused to [Gly-254 to Leu-290] of SEQ ID NO:40; [Met-1 to Lys-106] fused to [Leu-107 to Thr-134] fused to [Arg-166 to Gln-203] fused to [Val-204 to Ala-253] fused to [Gly-254 to Leu-290] of SEQ ID NO:40; or [Met-1 to Lys-106] fused to [Leu-107 to Thr-134] fused to [Glu-135 to Asn-165] fused to [Arg-166 to Gln-203] fused to [Gly-254 to Leu-290] of SEQ ID NO:40. Other combinations may include the polypeptide fragments in an order other than that recited above (e.g., [Met-1 to Lys-106] fused to [Arg-166 to Gln-203] fused to [Val-204 to Ala-253] fused to [Leu-107 to Thr-134] fused to [Gly-254 to Leu-290] of SEQ ID NO:40). Other combinations may also include heterologous polypeptide fragments as described herein and/or other polypeptides or polypeptide fragments of the present invention (e.g., [Met-1 to Lys-106] fused to [Glu-135 to Asn-165] fused to [Arg-166 to to Gln-202] fused to [Val-204 to Ala-253] fused to [Gly-254 to Leu-290] of SEQ ID NO:38 fused to a FLAG tag). Polynucleotides encoding any of these polypeptides are encompassed by the invention.

**[0258]** A further partial, non-limiting, and exemplary list of polypeptides of the invention which comprise, or alternatively consist of, combinations of amino acid sequences includes, for example, [Tyr-1 to Lys-47] fused to [Leu-48 to Thr-75] fused to [Val-76 to Lys-94] fused to [Gly-95 to Gln-132] fused to [Val-133 to Ala-182] fused to [Gly-183 to Leu-219] of SEQ ID NO:28; [Tyr-1 to Lys-47] fused to [Leu-48 to Thr-75] fused to [Val-76 to Lys-94] fused to [Val-133 to Ala-182] of SEQ ID NO:28; or [Tyr-1 to Lys-47] fused to [Val-76 to Lys-94] fused to [Val-133 to Ala-182] fused to [Gly-183 to Leu-219] of SEQ ID NO:28. Other combinations may include the polypeptide fragments in an order other than that recited above (e.g., [Tyr-1 to Lys-47] fused to [Gly-183 to Leu-219] fused to [Val-133 to Ala-182] fused to [Leu-48 to Thr-75] of SEQ ID NO:28). Other combinations may also include heterologous polypeptide fragments as described herein and/or other polypeptides or polypeptide fragments of the present invention (e.g., [Leu-48 to Thr-75] fused to [Val-76 to Lys-94] fused to [Gly-95 to Gln-132] fused to [Val-133 to Ala-182] of SEQ ID NO:28 fused to an Fc receptor tag). Polynucleotides encoding any of these polypeptides are encompassed by the invention.

**[0259]** A further partial, non-limiting, and exemplary list of polypeptides of the invention which comprise, or alternatively consist of, combinations of amino acid sequences includes, for example, [Tyr-1 to Lys-47] fused to [Leu-48 to Thr-75] fused to [Val-76 to Lys-94] fused to [Gly-95 to Gln-132] fused to [Val-133 to Ala-182] fused to [Gly-183 to Leu-219] of SEQ ID NO:30; [Tyr-1 to Lys-47] fused to [Leu-48 to Thr-75] fused to [Val-76 to Lys-94] fused to [Val-133 to Ala-182] of SEQ ID NO:30; or [Tyr-1 to Lys-47] fused to [Val-76 to Lys-94] fused to [Val-133 to Ala-182] fused to [Gly-183 to Leu-219] of SEQ ID NO:30. Other combinations may include the polypeptide fragments in an order other than that recited above (e.g., [Tyr-1 to Lys-47] fused to [Gly-183 to Leu-219] fused to [Val-133 to Ala-182] fused to [Leu-48 to Thr-75] of SEQ ID NO:30). Other combinations may also include heterologous polypeptide fragments as described herein and/or other polypeptides or polypeptide fragments of the present invention (e.g., [Leu-48 to Thr-75] fused to [Val-76 to Lys-94] fused to [Gly-95 to Gln-132] fused to [Val-133 to Ala-182] of SEQ ID NO:30 fused to an Fc receptor tag). Polynucleotides encoding any of these polypeptides are encompassed by the invention.

**[0260]** A further partial, non-limiting, and exemplary list of polypeptides of the invention which comprise, or alternatively consist of, combinations of amino acid sequences includes, for example, [Ala-1 to Thr-8] fused to [Val-9 to Lys-27] fused to [Gly-28 to Gln-65] fused to [Val-66 to Ala-115] fused to [Gly-116 to Leu-152] of SEQ ID NO:41; [Ala-1 to Thr-8] fused to [Gly-28 to Gln-65] fused to [Val-66 to Ala-115] fused to [Gly-116 to Leu-152] of SEQ ID NO:41; [Ala-1 to Thr-8] fused to [Val-9 to Lys-27] fused to [Gly-28 to Gln-65] fused to [Gly-116 to Leu-152] of SEQ ID NO:41; Other combinations may include the polypeptide fragments in an order other than that recited above (e.g. [Ala-1 to Thr-8] fused to [Gly-116 to Leu-152] fused to [Val-66 to Ala-115] fused to [Val-9 to Lys-27] of SEQ ID NO:41). Other combinations may also include heterologous polypeptide fragments as described herein and/or other polypeptides or polypeptide fragments of the present invention (e.g., [Ala-1 to Thr-8] fused to [Val-9 to Lys-27] fused to [Gly-28 to Gln-65] fused to [Val-66 to Ala-115] fused to [Gly-116 to Leu-152] of SEQ ID NO:41 fused to an Fc receptor tag). Polynucleotides encoding any of these polypeptides are encompassed by the invention.

**[0261]** A further partial, non-limiting, and exemplary list of polypeptides of the invention which comprise, or alternatively consist of, combinations of amino acid



sequences includes, for example, [Ala-1 to Thr-8] fused to [Glu-9 to Thr-40] fused to [Arg-41 to Gln-78] fused to [Val-79 to Ala-128] fused to [Gly-129 to Leu-165] of SEQ ID NO:42; [Ala-1 to Thr-8] fused to [Arg-41 to Gln-78] fused to [Val-79 to Ala-128] fused to [Gly-129 to Leu-165] of SEQ ID NO:42; [Ala-1 to Thr-8] fused to [Glu-9 to Thr-40] fused to [Arg-41 to Gln-78] fused to [Gly-129 to Leu-165] of SEQ ID NO:4. Other combinations may include the polypeptide fragments in an order other than that recited above (e.g. [Ala-1 to Thr-8] fused to [Gly-129 to Leu-165] fused to [Val-79 to Ala-128] fused to [Arg-41 to Gln-78] fused to [Glu-9 to Thr-40] of SEQ ID NO:42). Other combinations may also include heterologous polypeptide fragments as described herein and/or other polypeptides or polypeptide fragments of the present invention (e.g., [Ala-1 to Thr-8] fused to [Glu-9 to Thr-40] fused to [Arg-41 to Gln-78] fused to [Val-79 to Ala-128] fused to [Gly-129 to Leu-165] of SEQ ID NO:42 fused to an Fc receptor tag). Polynucleotides encoding any of these polypeptides are encompassed by the invention.

**[0262]** A further partial, non-limiting, and exemplary list of polypeptides of the invention which comprise, or alternatively consist of, combinations of amino acid sequences includes, for example, [Ala-1 to Thr-8] fused to [Glu-9 to Thr-40] fused to [Ile-41 to Lys-59] fused to [Gly-60 to Gln-97] fused to [Val-98 to Ala-147] fused to [Gly-148 to Leu-184] of SEQ ID NO:43; [Ala-1 to Thr-8] fused [Gly-60 to Gln-97] fused to [Gly-148 to Leu-184] of SEQ ID NO:43; [Ala-1 to Thr-8] fused to [Glu-9 to Thr-40] fused to [Gly-60 to Gln-97] fused to [Val-98 to Ala-147] fused to [Gly-148 to Leu-184] of SEQ ID NO:43; [Ala-1 to Thr-8] fused to [Ile-41 to Lys-59] fused to [Gly-60 to Gln-97] fused to [Val-98 to Ala-147] fused to [Gly-148 to Leu-184] of SEQ ID NO:43; or [Ala-1 to Thr-8] fused to [Glu-9 to Thr-40] fused to [Ile-41 to Lys-59] fused to [Gly-60 to Gln-97] fused to [Gly-148 to Leu-184] of SEQ ID NO:43; Other combinations may include the polypeptide fragments in an order other than that recited above (e.g., [Ala-1 to Thr-8] fused to [Gly-148 to Leu-184] fused to [Val-98 to Ala-147] fused to [Ile-41 to Lys-59] fused to [Glu-9 to Thr-40] fused to [Gly-60 to Gln-97] of SEQ ID NO:43). Other combinations may also include heterologous polypeptide fragments as described herein and/or other polypeptides or polypeptide fragments of the present invention (e.g., [Ala-1 to Thr-8] fused to [Glu-9 to Thr-40] fused to [Ile-41 to Lys-59] fused to [Val-98 to Ala-147] fused to [Gly-148 to Leu-184] of SEQ ID NO:43 fused to an Fc receptor tag). Polynucleotides encoding any of these polypeptides are encompassed by the invention.

**[0263]** Additional embodiments of the invention encompass Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide fragments comprising, or alternatively consisting of, functional regions of polypeptides of the invention, such as the Garnier-Robson  $\alpha$ -regions,  $\beta$ -regions, turn-regions, and coil-regions, Chou-Fasman  $\alpha$ -regions,  $\beta$ -regions, and coil-regions, Kyte-Doolittle hydrophilic regions and hydrophobic regions, Eisenberg  $\alpha$ - and  $\beta$ -amphipathic regions, Karplus-Schulz flexible regions, Emini surface-forming regions and Jameson-Wolf regions of high antigenic index set out in Figures 3 and 6 and in Table I and as described herein. In a preferred embodiment, the polypeptide fragments of the invention are antigenic. The data presented in columns VIII, IX, XIII, and XIV of Table I can be used to routinely determine regions of Neutrokin- $\alpha$  which exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from the data presented in columns VIII, IX, XIII, and/or IV by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response. Among highly preferred fragments of the invention are those that comprise regions of Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV that combine several structural features, such as several (e.g., 1, 2, 3 or 4) of the features set out above. Polynucleotides encoding these polypeptides are also encompassed by the invention.

**[0264]** In another embodiment, the invention provides a polypeptide comprising, or alternatively consisting of, an epitope-bearing portion of a polypeptide of the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, for instance, Geysen *et al.*, *Proc. Natl. Acad. Sci. USA* 81:3998- 4002 (1983).

**[0265]** As to the selection of polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are

routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, Sutcliffe, J. G., Shinnick, T. M., Green, N. and Learner, R. A. (1983) "Antibodies that react with predetermined sites on proteins", *Science*, 219:660-666. Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals. Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention. See, for instance, Wilson *et al.*, *Cell* 37:767-778 (1984) at 777.

[0266] Antigenic epitope-bearing peptides and polypeptides of the invention preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and, most preferably, between about 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof.

[0267] Non-limiting examples of antigenic polypeptides or peptides that can be used to generate Neutrokin- $\alpha$ - and/or Neutrokin- $\alpha$ SV-specific antibodies include: a polypeptide comprising, or alternatively consisting of, amino acid residues from about Phe-115 to about Leu-147 in Figures 1A and 1B (SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, amino acid residues from about Ile-150 to about Tyr-163 in Figures 1A and 1B (SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, amino acid residues from about Ser-171 to about Phe-194 in Figures 1A and 1B (SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, amino acid residues from about Glu-223 to about Tyr-246 in Figures 1A and 1B (SEQ ID NO:2); and a polypeptide comprising, or alternatively consisting of, amino acid residues from about Ser-271 to about Phe-278 in Figures 1A and 1B (SEQ ID NO:2). In this context, "about" means the particularly recited ranges and ranges larger or smaller by several, a few, 5, 4, 3, 2 or 1 amino acid residues at either or both the amino- and carboxy-

termini. These polypeptide fragments have been determined to bear antigenic epitopes of the Neutrokin- $\alpha$  polypeptide by the analysis of the Jameson-Wolf antigenic index, as shown in Figure 3 and Table I, above.

[0268] Non-limiting examples of antigenic polypeptides or peptides that can be used to generate Neutrokin- $\alpha$ - and/or Neutrokin- $\alpha$ SV-specific antibodies include: a polypeptide comprising, or alternatively consisting of, amino acid residues from about Pro-32 to about Leu-47 in Figures 5A and 5B (SEQ ID NO:19); a polypeptide comprising, or alternatively consisting of, amino acid residues from about Glu-116 to about Ser-143 in Figures 5A and 5B (SEQ ID NO:19); a polypeptide comprising, or alternatively consisting of, amino acid residues from about Phe-153 to about Tyr-173 in Figures 5A and 5B (SEQ ID NO:19); a polypeptide comprising, or alternatively consisting of, amino acid residues from about Pro-218 to about Tyr-227 in Figures 5A and 5B (SEQ ID NO:19); a polypeptide comprising, or alternatively consisting of, amino acid residues from about Ala-232 to about Gln-241 in Figures 5A and 5B (SEQ ID NO:19); a polypeptide comprising, or alternatively consisting of, amino acid residues from about Ile-244 to about Ala-249 in Figures 5A and 5B (SEQ ID NO:19); and a polypeptide comprising, or alternatively consisting of, amino acid residues from about Ser-252 to about Val-257 in Figures 5A and 5B (SEQ ID NO:19). In this context, "about" means the particularly recited ranges and ranges larger or smaller by several, a few, 5, 4, 3, 2 or 1 amino acid residues at either or both the amino- and carboxy-termini. Polynucleotides encoding these polypeptides are also encompassed by the invention. These polypeptide fragments have been determined to bear antigenic epitopes of the Neutrokin- $\alpha$ SV polypeptide by the analysis of the Jameson-Wolf antigenic index, as shown in Figure 6 and a tabular representation of the data presented in Figure 6 generated by the Protean component of the DNA\*STAR computer program (as set forth above).

[0269] The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means. *See, e.g.*, Houghten, R. A. (1985) General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids. *Proc. Natl. Acad. Sci. USA* 82:5131-5135; this "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U. S. Patent No. 4,631,211 to Houghten et al. (1986).

[0270] Epitope-bearing peptides and polypeptides of the invention have uses that include, but are not limited to, to induce antibodies according to methods well known in the art. See, for instance, Sutcliffe et al., *supra*; Wilson et al., *supra*; Chow, M. et al., *Proc. Natl. Acad. Sci. USA* 82:910-914; and Bittle, F. J. *et al.*, *J. Gen. Virol.* 66:2347-2354 (1985). Immunogenic epitope-bearing peptides of the invention, i.e., those parts of a protein that elicit an antibody response when the whole protein is the immunogen, are identified according to methods known in the art. See, for instance, Geysen et al., *supra*. Further still, U.S. Patent No. 5,194,392 to Geysen (1990) describes a general method of detecting or determining the sequence of monomers (amino acids or other compounds) which is a topological equivalent of the epitope (i.e., a "mimotope") which is complementary to a particular paratope (antigen binding site) of an antibody of interest. More generally, U.S. Patent No. 4,433,092 to Geysen (1989) describes a method of detecting or determining a sequence of monomers which is a topographical equivalent of a ligand which is complementary to the ligand binding site of a particular receptor of interest. Similarly, U.S. Patent No. 5,480,971 to Houghten, R. A. et al. (1996) on Peralkylated Oligopeptide Mixtures discloses linear C1-C7-alkyl peralkylated oligopeptides and sets and libraries of such peptides, as well as methods for using such oligopeptide sets and libraries for determining the sequence of a peralkylated oligopeptide that preferentially binds to an acceptor molecule of interest. Thus, non-peptide analogs of the epitope-bearing peptides of the invention also can be made routinely by these methods.

[0271] The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide having an amino acid sequence of SEQ ID NO:2, or an epitope of the polypeptide sequence encoded by a polynucleotide sequence contained in ATCC deposit No. 97768, or encoded by a polynucleotide that hybridizes to the complement of the sequence of SEQ ID NO:1 or the cDNA sequence contained in ATCC deposit No. 97768 (e.g., under hybridization conditions described herein). The present invention further encompasses polynucleotide sequences comprising, or alternatively consisting of, a sequence encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:1), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to the complementary strand (e.g., under hybridization conditions described herein).

[0272] The present invention also encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide having an amino acid sequence of SEQ ID NO:19, or an epitope of the polypeptide sequence encoded by a polynucleotide sequence contained in ATCC deposit No. 203518, or encoded by a polynucleotide that hybridizes to the complement of the sequence of SEQ ID NO:18 or the cDNA sequence contained in ATCC deposit No. 203518 (e.g., under hybridization conditions described herein). The present invention further encompasses polynucleotide sequences comprising, or alternatively consisting of, a sequence encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:18), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to the complementary strand (e.g., under hybridization conditions described herein).

[0273] The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies described *infra*. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998- 4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross- reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

[0274] Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985), further described in U.S. Patent No. 4,631,211).

[0275] In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and, most preferably, between about 15 to about 30 amino acids.

Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe et al., Science 219:660-666 (1983)).

[0276] Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., *supra*; Wilson et al., *supra*; Chow et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle et al., J. Gen. Virol. 66:2347-2354 (1985). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

[0277] Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, *in vivo* immunization, *in vitro* immunization, and phage display methods. See, e.g., Sutcliffe et al., *supra*; Wilson et al., *supra*, and Bittle et al., J. Gen. Virol., 66:2347-2354 (1985). If *in vivo* immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals

such as rabbits, rats and mice are immunized with either free or carrier-coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 micrograms of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

[0278] As one of skill in the art will appreciate, and as discussed above, the polypeptides of the present invention comprising an immunogenic or antigenic epitope can be fused to other polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof), or albumin (including but not limited to recombinant human albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)), resulting in chimeric polypeptides. Such fusion proteins may facilitate purification and may increase half-life *in vivo*. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., *Nature*, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion desulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., *J. Biochem.*, 270:3958-3964 (1995). Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of



the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, Proc. Natl. Acad. Sci. USA 88:8972- 897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix-binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto  $\text{Ni}^{2+}$  nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

[0279] In another embodiment, the Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides of the present invention and the epitope-bearing fragments thereof are fused with a heterologous antigen (e.g., polypeptide, carbohydrate, phospholipid, or nucleic acid). In specific embodiments, the heterologous antigen is an immunogen.

[0280] In a more specific embodiment, the heterologous antigen is the gp120 protein of HIV, or a fragment thereof. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0281] In another embodiment, the Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides of the present invention and the epitope-bearing fragments thereof are fused with polypeptide sequences of another TNF ligand family member (or biologically active fragments or variants thereof). In a specific embodiment, the Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides of the present invention are fused with a CD40L polypeptide sequence. In a preferred embodiment, the CD40L polypeptide sequence is soluble.

[0282] The techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate the activities of Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV thereby effectively generating agonists and antagonists of Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV. *See generally*, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458, and Patten, P. A., *et al.*, *Curr. Opinion Biotechnol.* 8:724-33 (1997); Harayama, S. *Trends Biotechnol.* 16(2):76-82 (1998); Hansson, L. O., *et al.*, *J. Mol. Biol.* 287:265-76 (1999); and Lorenzo, M. M. and Blasco, R. *Biotechniques* 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference). In

one embodiment, alteration of Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides and corresponding polypeptides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired Neutrokin-alpha and/or Neutrokin-alphaSV molecule by homologous, or site-specific, recombination. In another embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides and corresponding polypeptides may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of Neutrokin-alpha and/or Neutrokin-alphaSV may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules. In preferred embodiments, the heterologous molecules are, for example, TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), AIM-II (International Publication No. WO 97/34911), APRIL (J. Exp. Med. 188(6):1185-1190), endokine-alpha (International Publication No. WO 98/07880), OPG, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-1BB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892), TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), TR12, CAD, and v-FLIP. In further embodiments, the heterologous molecules are any member of the TNF family.

**[0283]** In a preferred embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides of the invention (including biologically active fragments or variants thereof), are fused with soluble CD40L polypeptides, or biologically active fragments or variants thereof.

**[0284]** In another preferred embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides of the invention (including biologically active fragments or variants

thereof), are fused with soluble APRIL polypeptides (e.g., SEQ ID NO:20 or SEQ ID NO:47), or biologically active fragments or variants thereof.

**[0285]** To improve or alter the characteristics of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides, protein engineering may be employed. Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or "muteins including single or multiple amino acid substitutions, deletions, additions or fusion proteins. Such modified polypeptides can show, e.g., enhanced activity or increased stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions. For instance, for many proteins, including the extracellular domain or the mature form(s) of a secreted protein, it is known in the art that one or more amino acids may be deleted from the N-terminus or C-terminus without substantial loss of biological function. For instance, Ron et al., J. Biol. Chem., 268:2984-2988 (1993) reported modified KGF proteins that had heparin binding activity even if 3, 8, or 27 amino-terminal amino acid residues were missing.

**[0286]** In the present case, since the protein of the invention is a member of the TNF polypeptide family, deletions of N-terminal amino acids up to the Gly (G) residue at position 191 in Figures 1A and 1B (SEQ ID NO:2) may retain some biological activity such as, for example, the ability to stimulate lymphocyte (e.g., B cell) proliferation, differentiation, and/or activation, and cytotoxicity to appropriate target cells. Polypeptides having further N-terminal deletions including the Gly (G) residue would not be expected to retain biological activities because it is known that this residue in TNF-related polypeptides is in the beginning of the conserved domain required for biological activities. However, even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the protein, other functional activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or extracellular domain of the protein generally will be retained when less than the majority of the residues of the complete or extracellular domain of the protein are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

[0287] Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the Neutrokin- $\alpha$  shown in Figures 1A and 1B (SEQ ID NO:2), up to the glycine residue at position 191 (Gly-191 residue from the amino terminus), and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues  $n^1$ -285 of SEQ ID NO:2, where  $n^1$  is an integer in the range of the amino acid position of amino acid residues 2-190 of the amino acid sequence in SEQ ID NO:2. Polynucleotides encoding these polypeptides are also encompassed by the invention. More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group consisting of residues 2-285, 3-285, 4-285, 5-285, 6-285, 7-285, 8-285, 9-285, 10-285, 11-285, 12-285, 13-285, 14-285, 15-285, 16-285, 17-285, 18-285, 19-285, 20-285, 21-285, 22-285, 23-285, 24-285, 25-285, 26-285, 27-285, 28-285, 29-285, 30-285, 31-285, 32-285, 33-285, 34-285, 35-285, 36-285, 37-285, 38-285, 39-285, 40-285, 41-285, 42-285, 43-285, 44-285, 45-285, 46-285, 47-285, 48-285, 49-285, 50-285, 51-285, 52-285, 53-285, 54-285, 55-285, 56-285, 57-285, 58-285, 59-285, 60-285, 61-285, 62-285, 63-285, 64-285, 65-285, 66-285, 67-285, 68-285, 69-285, 70-285, 71-285, 72-285, 73-285, 74-285, 75-285, 76-285, 77-285, 78-285, 79-285, 80-285, 81-285, 82-285, 83-285, 84-285, 85-285, 86-285, 87-285, 88-285, 89-285, 90-285, 91-285, 92-285, 93-285, 94-285, 95-285, 96-285, 97-285, 98-285, 99-285, 100-285, 101-285, 102-285, 103-285, 104-285, 105-285, 106-285, 107-285, 108-285, 109-285, 110-285, 111-285, 112-285, 113-285, 114-285, 115-285, 116-285, 117-285, 118-285, 119-285, 120-285, 121-285, 122-285, 123-285, 124-285, 125-285, 126-285, 127-285, 128-285, 129-285, 130-285, 131-285, 132-285, 133-285, 134-285, 135-285, 136-285, 137-285, 138-285, 139-285, 140-285, 141-285, 142-285, 143-285, 144-285, 145-285, 146-285, 147-285, 148-285, 149-285, 150-285, 151-285, 152-285, 153-285, 154-285, 155-285, 156-285, 157-285, 158-285, 159-285, 160-285, 161-285, 162-285, 163-285, 164-285, 165-285, 166-285, 167-285, 168-285, 169-285, 170-285, 171-285, 172-285, 173-285, 174-285, 175-285, 176-285, 177-285, 178-285, 179-285, 180-285, 181-285, 182-285, 183-285, 184-285, 185-285, 186-285, 187-285, 188-285, 189-285, and 190-285 of SEQ ID NO:2. Polypeptides encoded by these polynucleotides are also encompassed by the invention. The present

invention is also directed to nucleic acid molecules comprising, or alternatively, consisting of, a polynucleotide sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the polynucleotide sequence encoding the Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these nucleic acids and/or polynucleotide sequences are also encompassed by the invention, as are polypeptides comprising, or alternatively consisting of, an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence described above, and polynucleotides that encode such polypeptides.

**[0288]** Furthermore, since the predicted extracellular domain of the Neutrokin- $\alpha$  polypeptides of the invention may itself elicit biological activity, deletions of N- and C-terminal amino acid residues from the predicted extracellular region of the polypeptide (spanning positions Gln-73 to Leu-285 of SEQ ID NO:2) may retain some biological activity such as, for example, ligand binding, stimulation of lymphocyte (e.g., B cell) proliferation, differentiation, and/or activation, and modulation of cell replication or modulation of target cell activities. However, even if deletion of one or more amino acids from the N-terminus of the predicted extracellular domain of a Neutrokin- $\alpha$  polypeptide results in modification or loss of one or more biological functions of the polypeptide, other functional activities may still be retained. Thus, the ability of the shortened polypeptides to induce and/or bind to antibodies which recognize the complete or mature or extracellular domains of the polypeptides generally will be retained when less than the majority of the residues of the complete or mature or extracellular domains of the polypeptides are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

**[0289]** Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of Neutrokin- $\alpha$  shown in SEQ ID NO:2, up to the glycine residue at position number 280, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues  $n^2$ -285 of SEQ ID NO:2, where  $n^2$  is an integer in the range of the amino acid

position of amino acid residues 73-280 in SEQ ID NO:2, and 73 is the position of the first residue from the N-terminus of the predicted extracellular domain of the Neurokinine-alpha polypeptide (disclosed in SEQ ID NO:2). Polynucleotides encoding these polypeptides are also encompassed by the invention. More in particular, in certain embodiments, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group consisting of residues of Q-73 to L-285; G-74 to L-285; D-75 to L-285; L-76 to L-285; A-77 to L-285; S-78 to L-285; L-79 to L-285; R-80 to L-285; A-81 to L-285; E-82 to L-285; L-83 to L-285; Q-84 to L-285; G-85 to L-285; H-86 to L-285; H-87 to L-285; A-88 to L-285; E-89 to L-285; K-90 to L-285; L-91 to L-285; P-92 to L-285; A-93 to L-285; G-94 to L-285; A-95 to L-285; G-96 to L-285; A-97 to L-285; P-98 to L-285; K-99 to L-285; A-100 to L-285; G-101 to L-285; L-102 to L-285; E-103 to L-285; E-104 to L-285; A-105 to L-285; P-106 to L-285; A-107 to L-285; V-108 to L-285; T-109 to L-285; A-110 to L-285; G-111 to L-285; L-112 to L-285; K-113 to L-285; I-114 to L-285; F-115 to L-285; E-116 to L-285; P-117 to L-285; P-118 to L-285; A-119 to L-285; P-120 to L-285; G-121 to L-285; E-122 to L-285; G-123 to L-285; N-124 to L-285; S-125 to L-285; S-126 to L-285; Q-127 to L-285; N-128 to L-285; S-129 to L-285; R-130 to L-285; N-131 to L-285; K-132 to L-285; R-133 to L-285; A-134 to L-285; V-135 to L-285; Q-136 to L-285; G-137 to L-285; P-138 to L-285; E-139 to L-285; E-140 to L-285; T-141 to L-285; V-142 to L-285; T-143 to L-285; Q-144 to L-285; D-145 to L-285; C-146 to L-285; L-147 to L-285; Q-148 to L-285; L-149 to L-285; I-150 to L-285; A-151 to L-285; D-152 to L-285; S-153 to L-285; E-154 to L-285; T-155 to L-285; P-156 to L-285; T-157 to L-285; I-158 to L-285; Q-159 to L-285; K-160 to L-285; G-161 to L-285; S-162 to L-285; Y-163 to L-285; T-164 to L-285; F-165 to L-285; V-166 to L-285; P-167 to L-285; W-168 to L-285; L-169 to L-285; L-170 to L-285; S-171 to L-285; F-172 to L-285; K-173 to L-285; R-174 to L-285; G-175 to L-285; S-176 to L-285; A-177 to L-285; L-178 to L-285; E-179 to L-285; E-180 to L-285; K-181 to L-285; E-182 to L-285; N-183 to L-285; K-184 to L-285; I-185 to L-285; L-186 to L-285; V-187 to L-285; K-188 to L-285; E-189 to L-285; T-190 to L-285; G-191 to L-285; Y-192 to L-285; F-193 to L-285; F-194 to L-285; I-195 to L-285; Y-196 to L-285; G-197 to L-285; Q-198 to L-285; V-199 to L-285; L-200 to L-285; Y-201 to L-285; T-202 to L-285; D-203 to L-285; K-204 to L-285; T-205 to L-285; Y-206 to L-285; A-207 to L-285; M-208 to L-285; G-209 to L-285; H-210 to

L-285; L-211 to L-285; I-212 to L-285; Q-213 to L-285; R-214 to L-285; K-215 to L-285; K-216 to L-285; V-217 to L-285; H-218 to L-285; V-219 to L-285; F-220 to L-285; G-221 to L-285; D-222 to L-285; E-223 to L-285; L-224 to L-285; S-225 to L-285; L-226 to L-285; V-227 to L-285; T-228 to L-285; L-229 to L-285; F-230 to L-285; R-231 to L-285; C-232 to L-285; I-233 to L-285; Q-234 to L-285; N-235 to L-285; M-236 to L-285; P-237 to L-285; E-238 to L-285; T-239 to L-285; L-240 to L-285; P-241 to L-285; N-242 to L-285; N-243 to L-285; S-244 to L-285; C-245 to L-285; Y-246 to L-285; S-247 to L-285; A-248 to L-285; G-249 to L-285; I-250 to L-285; A-251 to L-285; K-252 to L-285; L-253 to L-285; E-254 to L-285; E-255 to L-285; G-256 to L-285; D-257 to L-285; E-258 to L-285; L-259 to L-285; Q-260 to L-285; L-261 to L-285; A-262 to L-285; I-263 to L-285; P-264 to L-285; R-265 to L-285; E-266 to L-285; N-267 to L-285; A-268 to L-285; Q-269 to L-285; I-270 to L-285; S-271 to L-285; L-272 to L-285; D-273 to L-285; G-274 to L-285; D-275 to L-285; V-276 to L-285; T-277 to L-285; F-278 to L-285; F-279 to L-285; and G-280 to L-285 of SEQ ID NO:2. Polypeptides encoded by these polynucleotides are also encompassed by the invention. The present invention is also directed to nucleic acid molecules comprising, or alternatively, consisting of, a polynucleotide sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the polynucleotide sequence encoding the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these nucleic acids and/or polynucleotide sequences are also encompassed by the invention, as are polypeptides comprising, or alternatively consisting of, an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence described above, and polynucleotides that encode such polypeptides.

[0290] Highly preferred embodiments of the invention are directed to nucleic acid molecules comprising, or alternatively consisting of a polynucleotide having a nucleotide sequence at least 80%, 85%, 90% identical and more preferably at least 95%, 96%, 97%, 98%, 99% or 100% identical to a polynucleotide sequence encoding the Neutrokin-alpha polypeptide having the amino acid sequence at positions 134-285 in Figures 1A and 1B (SEQ ID NO:2). Preferred embodiments of the invention are directed to nucleic acid molecules comprising, or alternatively consisting of a polynucleotide having a nucleotide

sequence at least 90% identical to a polynucleotide sequence encoding the Neutrokin-alpha polypeptide having the amino acid sequence at positions 134-285 in Figures 1A and 1B (SEQ ID NO:2). More preferred embodiments of the invention are directed to nucleic acid molecules comprising, or alternatively consisting of a polynucleotide having a nucleotide sequence at least 95% identical to a polynucleotide sequence encoding the Neutrokin-alpha polypeptide having the amino acid sequence at positions 134-285 in Figures 1A and 1B (SEQ ID NO:2). More preferred embodiments of the invention are directed to nucleic acid molecules comprising, or alternatively consisting of a polynucleotide having a nucleotide sequence at least 96% identical to a polynucleotide sequence encoding the Neutrokin-alpha polypeptide having the amino acid sequence at positions 134-285 in Figures 1A and 1B (SEQ ID NO:2).

[0291] Additionally, more preferred embodiments of the invention are directed to nucleic acid molecules comprising, or alternatively consisting of a polynucleotide having a nucleotide sequence at least 97% to a polynucleotide sequence encoding the Neutrokin-alpha polypeptide having the amino acid sequence at positions 134-285 in Figures 1A and 1B (SEQ ID NO:2). Additionally, more preferred embodiments of the invention are directed to nucleic acid molecules comprising, or alternatively consisting of a polynucleotide having a nucleotide sequence at least 98% to a polynucleotide sequence encoding the Neutrokin-alpha polypeptide having the amino acid sequence at positions 134-285 in Figures 1A and 1B (SEQ ID NO:2). Additionally, more preferred embodiments of the invention are directed to nucleic acid molecules comprising, or alternatively consisting of a polynucleotide having a nucleotide sequence at least 99% identical to a polynucleotide sequence encoding the Neutrokin-alpha polypeptide having the amino acid sequence at positions 134-285 in Figures 1A and 1B (SEQ ID NO:2).

[0292] In specific embodiments, a polypeptide comprising, or alternatively consisting of, one of the following N-terminally deleted polypeptide fragments of Neutrokin-alpha and/or Neutrokin-alphaSV are preferred: amino acid residues Ala-71 through Leu-285, amino acid residues Ala-81 through Leu-285, amino acid residues Leu-112 through Leu-285, amino acid residues Ala-134 through Leu-285, amino acid residues Leu-147 through Leu-285, and amino acid residues Gly-161 through Leu-285 of SEQ ID NO:2. Polynucleotides encoding these polypeptides are also encompassed by the invention.



[0293] Similarly, many examples of biologically functional C-terminal deletion muteins are known. For instance, Interferon gamma shows up to ten times higher activities by deleting 8-10 amino acid residues from the carboxy terminus of the protein (Döbeli et al., *J. Biotechnology* 7:199-216 (1988)). Since the present protein is a member of the TNF polypeptide family, deletions of C-terminal amino acids up to the leucine residue at position 284 are expected to retain most if not all biological activity such as, for example, ligand binding, the ability to stimulate lymphocyte (e.g., B cell) proliferation, differentiation, and/or activation, and modulation of cell replication. Polypeptides having deletions of up to about 10 additional C-terminal residues (i.e., up to the glycine residue at position 274) also may retain some activity such as receptor binding, although such polypeptides would lack a portion of the conserved TNF domain which extends to about Leu-284 of SEQ ID NO:2. However, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other functional activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or mature protein generally will be retained when less than the majority of the residues of the complete or mature protein are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

[0294] Accordingly, the present invention further provides polypeptides having one or more residues deleted from the carboxy terminus of the amino acid sequence of the Neutrokin- $\alpha$  polypeptide shown in Figures 1A and 1B (SEQ ID NO:2), up to the glycine residue at position 274 (Gly-274) and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues 1- $m^1$  of the amino acid sequence in SEQ ID NO:2, where  $m^1$  is any integer in the range of the amino acid position of amino acid residues 274-284 in SEQ ID NO:2. Polynucleotides encoding these polypeptides are also encompassed by the invention. More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group consisting of residues 1-274, 1-275, 1-276, 1-277, 1-278, 1-279, 1-280, 1-281, 1-282, 1-283 and 1-284 of SEQ ID NO:2.

Polypeptides encoded by these polynucleotides are also encompassed by the invention. The present invention is also directed to nucleic acid molecules comprising, or alternatively, consisting of, a polynucleotide sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the polynucleotide sequence encoding the Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these nucleic acids and/or polynucleotide sequences are also encompassed by the invention, as are polypeptides comprising, or alternatively consisting of, an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence described above, and polynucleotides that encode such polypeptides.

[0295] Also provided are polypeptides comprising, or alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues  $n^1$ - $m^1$  of SEQ ID NO:2, where  $n^1$  and  $m^1$  are integers as defined above. Also included are a nucleotide sequence encoding a polypeptide comprising, or alternatively consisting of, a portion of the complete Neutrokin- $\alpha$  amino acid sequence encoded by the deposited cDNA clone contained in ATCC Accession No. 97768 where this portion excludes from 1 to 190 amino acids from the amino terminus or from 1 to 11 amino acids from the C-terminus of the complete amino acid sequence (or any combination of these N-terminal and C-terminal deletions) encoded by the cDNA clone in the deposited plasmid. Polynucleotides encoding all of the above deletion polypeptides are encompassed by the invention.

[0296] Similarly, deletions of C-terminal amino acid residues of the predicted extracellular domain of Neutrokin- $\alpha$  up to the leucine residue at position 79 of SEQ ID NO:2 may retain some biological activity, such as, for example, ligand binding, stimulation of lymphocyte (e.g., B cell) proliferation, differentiation, and/or activation, and modulation of cell replication or modulation of target cell activities. Polypeptides having further C-terminal deletions including Leu-79 of SEQ ID NO:2 would not be expected to retain biological activities.

[0297] However, even if deletion of one or more amino acids from the C-terminus of a polypeptide results in modification or loss of one or more biological functions of the polypeptide, other functional activities may still be retained. Thus, the ability of the

shortened polypeptide to induce and/or bind to antibodies which recognize the complete, mature or extracellular forms of the polypeptide generally will be retained when less than the majority of the residues of the complete, mature or extracellular forms of the polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of the predicted extracellular domain retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

**[0298]** Accordingly, the present invention further provides polypeptides having one or more residues deleted from the carboxy terminus of the amino acid sequence of the predicted extracellular domain of Neutrokin- $\alpha$  polypeptide shown in SEQ ID NO:2, up to the leucine residue at position 79 of SEQ ID NO:2, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues 73-m<sup>2</sup> of the amino acid sequence in SEQ ID NO:2, where m<sup>2</sup> is any integer in the range of the amino acid position of amino acid residues 79 to 285 in the amino acid sequence in SEQ ID NO:2, and residue 78 is the position of the first residue at the C- terminus of the predicted extracellular domain of the Neutrokin- $\alpha$  polypeptide (disclosed in SEQ ID NO:2). Polypeptides encoded by these polynucleotides are also encompassed by the invention. More in particular, in certain embodiments, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group consisting of residues Q-73 to Leu-285; Q-73 to L-284; Q-73 to K-283; Q-73 to L-282; Q-73 to A-281; Q-73 to G-280; Q-73 to F-279; Q-73 to F-278; Q-73 to T-277; Q-73 to V-276; Q-73 to D-275; Q-73 to G-274; Q-73 to D-273; Q-73 to L-272; Q-73 to S-271; Q-73 to I-270; Q-73 to Q-269; Q-73 to A-268; Q-73 to N-267; Q-73 to E-266; Q-73 to R-265; Q-73 to P-264; Q-73 to I-263; Q-73 to A-262; Q-73 to L-261; Q-73 to Q-260; Q-73 to L-259; Q-73 to E-258; Q-73 to D-257; Q-73 to G-256; Q-73 to E-255; Q-73 to E-254; Q-73 to L-253; Q-73 to K-252; Q-73 to A-251; Q-73 to I-250; Q-73 to G-249; Q-73 to A-248; Q-73 to S-247; Q-73 to Y-246; Q-73 to C-245; Q-73 to S-244; Q-73 to N-243; Q-73 to N-242; Q-73 to P-241; Q-73 to L-240; Q-73 to T-239; Q-73 to E-238; Q-73 to P-237; Q-73 to M-236; Q-73 to N-235; Q-73 to Q-234; Q-73 to I-233; Q-73 to C-232; Q-73 to R-231; Q-73 to F-230; Q-73 to L-229; Q-73 to T-228; Q-73 to V-227; Q-73 to L-226; Q-73 to S-225; Q-73 to L-224; Q-73 to E-223; Q-73 to D-222;

Q-73 to G-221; Q-73 to F-220; Q-73 to V-219; Q-73 to H-218; Q-73 to V-217; Q-73 to K-216; Q-73 to K-215; Q-73 to R-214; Q-73 to Q-213; Q-73 to I-212; Q-73 to L-211; Q-73 to H-210; Q-73 to G-209; Q-73 to M-208; Q-73 to A-207; Q-73 to Y-206; Q-73 to T-205; Q-73 to K-204; Q-73 to D-203; Q-73 to T-202; Q-73 to Y-201; Q-73 to L-200; Q-73 to V-199; Q-73 to Q-198; Q-73 to G-197; Q-73 to Y-196; Q-73 to I-195; Q-73 to F-194; Q-73 to F-193; Q-73 to Y-192; Q-73 to G-191; Q-73 to T-190; Q-73 to E-189; Q-73 to K-188; Q-73 to V-187; Q-73 to L-186; Q-73 to I-185; Q-73 to K-184; Q-73 to N-183; Q-73 to E-182; Q-73 to K-181; Q-73 to E-180; Q-73 to E-179; Q-73 to L-178; Q-73 to A-177; Q-73 to S-176; Q-73 to G-175; Q-73 to R-174; Q-73 to K-173; Q-73 to F-172; Q-73 to S-171; Q-73 to L-170; Q-73 to L-169; Q-73 to W-168; Q-73 to P-167; Q-73 to V-166; Q-73 to F-165; Q-73 to T-164; Q-73 to Y-163; Q-73 to S-162; Q-73 to G-161; Q-73 to K-160; Q-73 to Q-159; Q-73 to I-158; Q-73 to T-157; Q-73 to P-156; Q-73 to T-155; Q-73 to E-154; Q-73 to S-153; Q-73 to D-152; Q-73 to A-151; Q-73 to I-150; Q-73 to L-149; Q-73 to Q-148; Q-73 to L-147; Q-73 to C-146; Q-73 to D-145; Q-73 to Q-144; Q-73 to T-143; Q-73 to V-142; Q-73 to T-141; Q-73 to E-140; Q-73 to E-139; Q-73 to P-138; Q-73 to G-137; Q-73 to Q-136; Q-73 to V-135; Q-73 to A-134; Q-73 to R-133; Q-73 to K-132; Q-73 to N-131; Q-73 to R-130; Q-73 to S-129; Q-73 to N-128; Q-73 to Q-127; Q-73 to S-126; Q-73 to S-125; Q-73 to N-124; Q-73 to G-123; Q-73 to E-122; Q-73 to G-121; Q-73 to P-120; Q-73 to A-119; Q-73 to P-118; Q-73 to P-117; Q-73 to E-116; Q-73 to F-115; Q-73 to I-114; Q-73 to K-113; Q-73 to L-112; Q-73 to G-111; Q-73 to A-110; Q-73 to T-109; Q-73 to V-108; Q-73 to A-107; Q-73 to P-106; Q-73 to A-105; Q-73 to E-104; Q-73 to E-103; Q-73 to L-102; Q-73 to G-101; Q-73 to A-100; Q-73 to K-99; Q-73 to P-98; Q-73 to A-97; Q-73 to G-96; Q-73 to A-95; Q-73 to G-94; Q-73 to A-93; Q-73 to P-92; Q-73 to L-91; Q-73 to K-90; Q-73 to E-89; Q-73 to A-88; Q-73 to H-87; Q-73 to H-86; Q-73 to G-85; Q-73 to Q-84; Q-73 to L-83; Q-73 to E-82; Q-73 to A-81; Q-73 to R-80; and Q-73 to L-79 of SEQ ID NO:2. Polypeptides encoded by these polynucleotides are also encompassed by the invention. The present invention is also directed to nucleic acid molecules comprising, or alternatively, consisting of, a polynucleotide sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the polynucleotide sequence encoding the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide

sequence. Polypeptides encoded by these nucleic acids and/or polynucleotide sequences are also encompassed by the invention, as are polypeptides comprising, or alternatively consisting of, an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence described above, and polynucleotides that encode such polypeptides.

[0299] The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini of the predicted extracellular domain of Neutrokin- $\alpha$ , which may be described generally as having residues  $n^2$ - $m^2$  of SEQ ID NO:2 where  $n^2$  and  $m^2$  are integers as defined above.

[0300] In another embodiment, a nucleotide sequence encoding a polypeptide consisting of a portion of the extracellular domain of the Neutrokin- $\alpha$  amino acid sequence encoded by the cDNA plasmid contained in the deposit having ATCC accession no. 97768, where this portion excludes from 1 to about 206 amino acids from the amino terminus of the extracellular domain of the amino acid sequence encoded by the cDNA plasmid contained in the deposit having ATCC accession no. 97768, or from 1 to about 206 amino acids from the carboxy terminus of the extracellular domain of the amino acid sequence encoded by the cDNA plasmid contained in the deposit having ATCC accession no. 97768, or any combination of the above amino terminal and carboxy terminal deletions, of the entire extracellular domain of the amino acid sequence encoded by the cDNA plasmid contained in the deposit having ATCC accession no. 97768.

[0301] As mentioned above, even if deletion of one or more amino acids from the N-terminus of a polypeptide results in modification or loss of one or more functional activities (e.g., biological activity) of the polypeptide, other functions or biological activities may still be retained. Thus, the ability of a shortened Neutrokin- $\alpha$  mutein to induce and/or bind to antibodies which recognize the full-length or mature forms or the extracellular domain of the polypeptide generally will be retained when less than the majority of the residues of the full-length or mature or extracellular domain of the polypeptide are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a Neutrokin- $\alpha$  mutein with a large number of deleted N-terminal amino acid residues may retain some functional (e.g., biological or

immunogenic) activities. In fact, peptides composed of as few as six Neutrokin- $\alpha$  amino acid residues may often evoke an immune response.

**[0302]** Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the predicted full-length amino acid sequence of the Neutrokin- $\alpha$  shown in SEQ ID NO:2, up to the glycine residue at position number 280 of the sequence shown SEQ ID NO:2 and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues  $n^3$ -285 of the sequence shown in SEQ ID NO:2, where  $n^3$  is an integer in the range of the amino acid position of amino acid residues 1 to 280 of the amino acid sequence in SEQ ID NO:2.

**[0303]** More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group consisting of residues of D-2 to L-285; D-3 to L-285; S-4 to L-285; T-5 to L-285; E-6 to L-285; R-7 to L-285; E-8 to L-285; Q-9 to L-285; S-10 to L-285; R-11 to L-285; L-12 to L-285; T-13 to L-285; S-14 to L-285; C-15 to L-285; L-16 to L-285; K-17 to L-285; K-18 to L-285; R-19 to L-285; E-20 to L-285; E-21 to L-285; M-22 to L-285; K-23 to L-285; L-24 to L-285; K-25 to L-285; E-26 to L-285; C-27 to L-285; V-28 to L-285; S-29 to L-285; I-30 to L-285; L-31 to L-285; P-32 to L-285; R-33 to L-285; K-34 to L-285; E-35 to L-285; S-36 to L-285; P-37 to L-285; S-38 to L-285; V-39 to L-285; R-40 to L-285; S-41 to L-285; S-42 to L-285; K-43 to L-285; D-44 to L-285; G-45 to L-285; K-46 to L-285; L-47 to L-285; L-48 to L-285; A-49 to L-285; A-50 to L-285; T-51 to L-285; L-52 to L-285; L-53 to L-285; L-54 to L-285; A-55 to L-285; L-56 to L-285; L-57 to L-285; S-58 to L-285; C-59 to L-285; C-60 to L-285; L-61 to L-285; T-62 to L-285; V-63 to L-285; V-64 to L-285; S-65 to L-285; F-66 to L-285; Y-67 to L-285; Q-68 to L-285; V-69 to L-285; A-70 to L-285; A-71 to L-285; L-72 to L-285; Q-73 to L-285; G-74 to L-285; D-75 to L-285; L-76 to L-285; A-77 to L-285; S-78 to L-285; L-79 to L-285; R-80 to L-285; A-81 to L-285; E-82 to L-285; L-83 to L-285; Q-84 to L-285; G-85 to L-285; H-86 to L-285; H-87 to L-285; A-88 to L-285; E-89 to L-285; K-90 to L-285; L-91 to L-285; P-92 to L-285; A-93 to L-285; G-94 to L-285; A-95 to L-285; G-96 to L-285; A-97 to L-285; P-98 to L-285; K-99 to L-285; A-100 to L-285; G-101 to L-285; L-102 to L-285; E-103 to L-285; E-104 to L-285; A-105 to L-285; P-106 to L-285; A-107 to L-285; V-108 to L-285; T-109 to L-285; A-110 to L-285; G-111 to L-285; L-112 to

L-285; K-113 to L-285; I-114 to L-285; F-115 to L-285; E-116 to L-285; P-117 to L-285; P-118 to L-285; A-119 to L-285; P-120 to L-285; G-121 to L-285; E-122 to L-285; G-123 to L-285; N-124 to L-285; S-125 to L-285; S-126 to L-285; Q-127 to L-285; N-128 to L-285; S-129 to L-285; R-130 to L-285; N-131 to L-285; K-132 to L-285; R-133 to L-285; A-134 to L-285; V-135 to L-285; Q-136 to L-285; G-137 to L-285; P-138 to L-285; E-139 to L-285; E-140 to L-285; T-141 to L-285; V-142 to L-285; T-143 to L-285; Q-144 to L-285; D-145 to L-285; C-146 to L-285; L-147 to L-285; Q-148 to L-285; L-149 to L-285; I-150 to L-285; A-151 to L-285; D-152 to L-285; S-153 to L-285; E-154 to L-285; T-155 to L-285; P-156 to L-285; T-157 to L-285; I-158 to L-285; Q-159 to L-285; K-160 to L-285; G-161 to L-285; S-162 to L-285; Y-163 to L-285; T-164 to L-285; F-165 to L-285; V-166 to L-285; P-167 to L-285; W-168 to L-285; L-169 to L-285; L-170 to L-285; S-171 to L-285; F-172 to L-285; K-173 to L-285; R-174 to L-285; G-175 to L-285; S-176 to L-285; A-177 to L-285; L-178 to L-285; E-179 to L-285; E-180 to L-285; K-181 to L-285; E-182 to L-285; N-183 to L-285; K-184 to L-285; I-185 to L-285; L-186 to L-285; V-187 to L-285; K-188 to L-285; E-189 to L-285; T-190 to L-285; G-191 to L-285; Y-192 to L-285; F-193 to L-285; F-194 to L-285; I-195 to L-285; Y-196 to L-285; G-197 to L-285; Q-198 to L-285; V-199 to L-285; L-200 to L-285; Y-201 to L-285; T-202 to L-285; D-203 to L-285; K-204 to L-285; T-205 to L-285; Y-206 to L-285; A-207 to L-285; M-208 to L-285; G-209 to L-285; H-210 to L-285; L-211 to L-285; I-212 to L-285; Q-213 to L-285; R-214 to L-285; K-215 to L-285; K-216 to L-285; V-217 to L-285; H-218 to L-285; V-219 to L-285; F-220 to L-285; G-221 to L-285; D-222 to L-285; E-223 to L-285; L-224 to L-285; S-225 to L-285; L-226 to L-285; V-227 to L-285; T-228 to L-285; L-229 to L-285; F-230 to L-285; R-231 to L-285; C-232 to L-285; I-233 to L-285; Q-234 to L-285; N-235 to L-285; M-236 to L-285; P-237 to L-285; E-238 to L-285; T-239 to L-285; L-240 to L-285; P-241 to L-285; N-242 to L-285; N-243 to L-285; S-244 to L-285; C-245 to L-285; Y-246 to L-285; S-247 to L-285; A-248 to L-285; G-249 to L-285; I-250 to L-285; A-251 to L-285; K-252 to L-285; L-253 to L-285; E-254 to L-285; E-255 to L-285; G-256 to L-285; D-257 to L-285; E-258 to L-285; L-259 to L-285; Q-260 to L-285; L-261 to L-285; A-262 to L-285; I-263 to L-285; P-264 to L-285; R-265 to L-285; E-266 to L-285; N-267 to L-285; A-268 to L-285; Q-269 to L-285; I-270 to L-285; S-271 to L-285; L-272 to L-285; D-273 to L-285; G-274 to L-285; D-275 to L-285; V-276 to L-285; T-277 to L-285; F-278 to L-285; F-279 to L-285; and G-280 to

L-285 of SEQ ID NO:2. The present application is also directed to nucleic acid molecules comprising, or alternatively, consisting of, a polynucleotide sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the polynucleotide sequence encoding the Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these nucleic acids and/or polynucleotide sequences are also encompassed by the invention, as are polypeptides comprising an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence described above, and polynucleotides that encode such polypeptides.

[0304] Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more functional activities (e.g., biological activity) of the protein, other functional activities may still be retained. Thus, the ability of a shortened Neutrokin- $\alpha$  mutein to induce and/or bind to antibodies which recognize the complete or mature form or the extracellular domain of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature form or the extracellular domain of the polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a Neutrokin- $\alpha$  mutein with a large number of deleted C-terminal amino acid residues may retain some functional (e.g., biological or immunogenic) activities. In fact, peptides composed of as few as six Neutrokin- $\alpha$  amino acid residues may often evoke an immune response.

[0305] Accordingly, the present invention further provides in another embodiment, polypeptides having one or more residues deleted from the carboxy terminus of the amino acid sequence of the Neutrokin- $\alpha$  shown in SEQ ID NO:2, up to the glutamic acid residue at position number 6, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues 1- $m^3$  of SEQ ID NO:2, where  $m^3$  is an integer in the range of the amino acid position of amino acid residues 6-284 of the amino acid sequence in SEQ ID NO:2.



[0306] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group consisting of residues M-1 to L-284; M-1 to K-283; M-1 to L-282; M-1 to A-281; M-1 to G-280; M-1 to F-279; M-1 to F-278; M-1 to T-277; M-1 to V-276; M-1 to D-275; M-1 to G-274; M-1 to D-273; M-1 to L-272; M-1 to S-271; M-1 to I-270; M-1 to Q-269; M-1 to A-268; M-1 to N-267; M-1 to E-266; M-1 to R-265; M-1 to P-264; M-1 to I-263; M-1 to A-262; M-1 to L-261; M-1 to Q-260; M-1 to L-259; M-1 to E-258; M-1 to D-257; M-1 to G-256; M-1 to E-255; M-1 to E-254; M-1 to L-253; M-1 to K-252; M-1 to A-251; M-1 to I-250; M-1 to G-249; M-1 to A-248; M-1 to S-247; M-1 to Y-246; M-1 to C-245; M-1 to S-244; M-1 to N-243; M-1 to N-242; M-1 to P-241; M-1 to L-240; M-1 to T-239; M-1 to E-238; M-1 to P-237; M-1 to M-236; M-1 to N-235; M-1 to Q-234; M-1 to I-233; M-1 to C-232; M-1 to R-231; M-1 to F-230; M-1 to L-229; M-1 to T-228; M-1 to V-227; M-1 to L-226; M-1 to S-225; M-1 to L-224; M-1 to E-223; M-1 to D-222; M-1 to G-221; M-1 to F-220; M-1 to V-219; M-1 to H-218; M-1 to V-217; M-1 to K-216; M-1 to K-215; M-1 to R-214; M-1 to Q-213; M-1 to I-212; M-1 to L-211; M-1 to H-210; M-1 to G-209; M-1 to M-208; M-1 to A-207; M-1 to Y-206; M-1 to T-205; M-1 to K-204; M-1 to D-203; M-1 to T-202; M-1 to Y-201; M-1 to L-200; M-1 to V-199; M-1 to Q-198; M-1 to G-197; M-1 to Y-196; M-1 to I-195; M-1 to F-194; M-1 to F-193; M-1 to Y-192; M-1 to G-191; M-1 to T-190; M-1 to E-189; M-1 to K-188; M-1 to V-187; M-1 to L-186; M-1 to I-185; M-1 to K-184; M-1 to N-183; M-1 to E-182; M-1 to K-181; M-1 to E-180; M-1 to E-179; M-1 to L-178; M-1 to A-177; M-1 to S-176; M-1 to G-175; M-1 to R-174; M-1 to K-173; M-1 to F-172; M-1 to S-171; M-1 to L-170; M-1 to L-169; M-1 to W-168; M-1 to P-167; M-1 to V-166; M-1 to F-165; M-1 to T-164; M-1 to Y-163; M-1 to S-162; M-1 to G-161; M-1 to K-160; M-1 to Q-159; M-1 to I-158; M-1 to T-157; M-1 to P-156; M-1 to T-155; M-1 to E-154; M-1 to S-153; M-1 to D-152; M-1 to A-151; M-1 to I-150; M-1 to L-149; M-1 to Q-148; M-1 to L-147; M-1 to C-146; M-1 to D-145; M-1 to Q-144; M-1 to T-143; M-1 to V-142; M-1 to T-141; M-1 to E-140; M-1 to E-139; M-1 to P-138; M-1 to G-137; M-1 to Q-136; M-1 to V-135; M-1 to A-134; M-1 to R-133; M-1 to K-132; M-1 to N-131; M-1 to R-130; M-1 to S-129; M-1 to N-128; M-1 to Q-127; M-1 to S-126; M-1 to S-125; M-1 to N-124; M-1 to G-123; M-1 to E-122; M-1 to G-121; M-1 to P-120; M-1 to A-119; M-1 to P-118; M-1 to P-117; M-1 to E-116; M-1 to F-115; M-1 to I-114; M-1 to K-113; M-1 to L-112; M-1 to G-111; M-1 to A-110; M-1 to T-109; M-1 to V-108; M-1 to

A-107; M-1 to P-106; M-1 to A-105; M-1 to E-104; M-1 to E-103; M-1 to L-102; M-1 to G-101; M-1 to A-100; M-1 to K-99; M-1 to P-98; M-1 to A-97; M-1 to G-96; M-1 to A-95; M-1 to G-94; M-1 to A-93; M-1 to P-92; M-1 to L-91; M-1 to K-90; M-1 to E-89; M-1 to A-88; M-1 to H-87; M-1 to H-86; M-1 to G-85; M-1 to Q-84; M-1 to L-83; M-1 to E-82; M-1 to A-81; M-1 to R-80; M-1 to L-79; M-1 to S-78; M-1 to A-77; M-1 to L-76; M-1 to D-75; M-1 to G-74; M-1 to Q-73; M-1 to L-72; M-1 to A-71; M-1 to A-70; M-1 to V-69; M-1 to Q-68; M-1 to Y-67; M-1 to F-66; M-1 to S-65; M-1 to V-64; M-1 to V-63; M-1 to T-62; M-1 to L-61; M-1 to C-60; M-1 to C-59; M-1 to S-58; M-1 to L-57; M-1 to L-56; M-1 to A-55; M-1 to L-54; M-1 to L-53; M-1 to L-52; M-1 to T-51; M-1 to A-50; M-1 to A-49; M-1 to L-48; M-1 to L-47; M-1 to K-46; M-1 to G-45; M-1 to D-44; M-1 to K-43; M-1 to S-42; M-1 to S-41; M-1 to R-40; M-1 to V-39; M-1 to S-38; M-1 to P-37; M-1 to S-36; M-1 to E-35; M-1 to K-34; M-1 to R-33; M-1 to P-32; M-1 to L-31; M-1 to I-30; M-1 to S-29; M-1 to V-28; M-1 to C-27; M-1 to E-26; M-1 to K-25; M-1 to L-24; M-1 to K-23; M-1 to M-22; M-1 to E-21; M-1 to E-20; M-1 to R-19; M-1 to K-18; M-1 to K-17; M-1 to L-16; M-1 to C-15; M-1 to S-14; M-1 to T-13; M-1 to L-12; M-1 to R-11; M-1 to S-10; M-1 to Q-9; M-1 to E-8; M-1 to R-7; and M-1 to E-6 of SEQ ID NO:2. The present application is also directed to nucleic acid molecules comprising, or alternatively, consisting of, a polynucleotide sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the polynucleotide sequence encoding the Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these nucleic acids and/or polynucleotide sequences are also encompassed by the invention, as are polypeptides comprising an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence described above, and polynucleotides that encode such polypeptides.

[0307] The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini of a Neutrokin- $\alpha$  polypeptide, which may be described generally as having residues  $n^3$ - $m^3$  of SEQ ID NO:2, where  $n^3$  and  $m^3$  are integers as defined above.

[0308] Furthermore, since the predicted extracellular domain of the Neutrokin- $\alpha$ SV polypeptides of the invention may itself elicit functional activity (e.g., biological activity), deletions of N- and C-terminal amino acid residues from the

predicted extracellular region of the polypeptide at positions Gln-73 to Leu-266 of SEQ ID NO:19 may retain some functional activity, such as, for example, ligand binding, to stimulation of lymphocyte (e.g., B cell) proliferation, differentiation, and/or activation, modulation of cell replication, modulation of target cell activities and/or immunogenicity. However, even if deletion of one or more amino acids from the N-terminus of the predicted extracellular domain of a Neutrokine-alphaSV polypeptide results in modification or loss of one or more functional activities of the polypeptide, other functional activities may still be retained. Thus, the ability of the shortened polypeptides to induce and/or bind to antibodies which recognize the complete or mature or extracellular domains of the polypeptides generally will be retained when less than the majority of the residues of the complete or mature or extracellular domains of the polypeptides are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

[0309] Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of Neutrokine-alphaSV shown in SEQ ID NO:19, up to the glycine residue at position number 261, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues  $n^4$ -266 of SEQ ID NO:19, where  $n^4$  is an integer in the range of the amino acid position of amino acid residues 73-261 of the amino acid sequence in SEQ ID NO:19, and 261 is the position of the first residue from the N-terminus of the predicted extracellular domain Neutrokine-alphaSV polypeptide (shown in SEQ ID NO:19).

[0310] More in particular, in certain embodiments, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group consisting of residues of Q-73 to L-266; G-74 to L-266; D-75 to L-266; L-76 to L-266; A-77 to L-266; S-78 to L-266; L-79 to L-266; R-80 to L-266; A-81 to L-266; E-82 to L-266; L-83 to L-266; Q-84 to L-266; G-85 to L-266; H-86 to L-266; H-87 to L-266; A-88 to L-266; E-89 to L-266; K-90 to L-266; L-91 to L-266; P-92 to L-266; A-93 to L-266; G-94 to L-266; A-95 to L-266; G-96 to L-266; A-97 to L-266; P-98 to L-266; K-99 to L-266; A-100 to L-266; G-101 to L-266; L-102 to L-266; E-103 to L-266; E-104 to L-266; A-105 to L-266; P-106 to L-266; A-107

to L-266; V-108 to L-266; T-109 to L-266; A-110 to L-266; G-111 to L-266; L-112 to L-266; K-113 to L-266; I-114 to L-266; F-115 to L-266; E-116 to L-266; P-117 to L-266; P-118 to L-266; A-119 to L-266; P-120 to L-266; G-121 to L-266; E-122 to L-266; G-123 to L-266; N-124 to L-266; S-125 to L-266; S-126 to L-266; Q-127 to L-266; N-128 to L-266; S-129 to L-266; R-130 to L-266; N-131 to L-266; K-132 to L-266; R-133 to L-266; A-134 to L-266; V-135 to L-266; Q-136 to L-266; G-137 to L-266; P-138 to L-266; E-139 to L-266; E-140 to L-266; T-141 to L-266; G-142 to L-266; S-143 to L-266; Y-144 to L-266; T-145 to L-266; F-146 to L-266; V-147 to L-266; P-148 to L-266; W-149 to L-266; L-150 to L-266; L-151 to L-266; S-152 to L-266; F-153 to L-266; K-154 to L-266; R-155 to L-266; G-156 to L-266; S-157 to L-266; A-158 to L-266; L-159 to L-266; E-160 to L-266; E-161 to L-266; K-162 to L-266; E-163 to L-266; N-164 to L-266; K-165 to L-266; I-166 to L-266; L-167 to L-266; V-168 to L-266; K-169 to L-266; E-170 to L-266; T-171 to L-266; G-172 to L-266; Y-173 to L-266; F-174 to L-266; F-175 to L-266; I-176 to L-266; Y-177 to L-266; G-178 to L-266; Q-179 to L-266; V-180 to L-266; L-181 to L-266; Y-182 to L-266; T-183 to L-266; D-184 to L-266; K-185 to L-266; T-186 to L-266; Y-187 to L-266; A-188 to L-266; M-189 to L-266; G-190 to L-266; H-191 to L-266; L-192 to L-266; I-193 to L-266; Q-194 to L-266; R-195 to L-266; K-196 to L-266; K-197 to L-266; V-198 to L-266; H-199 to L-266; V-200 to L-266; F-201 to L-266; G-202 to L-266; D-203 to L-266; E-204 to L-266; L-205 to L-266; S-206 to L-266; L-207 to L-266; V-208 to L-266; T-209 to L-266; L-210 to L-266; F-211 to L-266; R-212 to L-266; C-213 to L-266; I-214 to L-266; Q-215 to L-266; N-216 to L-266; M-217 to L-266; P-218 to L-266; E-219 to L-266; T-220 to L-266; L-221 to L-266; P-222 to L-266; N-223 to L-266; N-224 to L-266; S-225 to L-266; C-226 to L-266; Y-227 to L-266; S-228 to L-266; A-229 to L-266; G-230 to L-266; I-231 to L-266; A-232 to L-266; K-233 to L-266; L-234 to L-266; E-235 to L-266; E-236 to L-266; G-237 to L-266; D-238 to L-266; E-239 to L-266; L-240 to L-266; Q-241 to L-266; L-242 to L-266; A-243 to L-266; I-244 to L-266; P-245 to L-266; R-246 to L-266; E-247 to L-266; N-248 to L-266; A-249 to L-266; Q-250 to L-266; I-251 to L-266; S-252 to L-266; L-253 to L-266; D-254 to L-266; G-255 to L-266; D-256 to L-266; V-257 to L-266; T-258 to L-266; F-259 to L-266; F-260 to L-266; and G-261 to L-266 of SEQ ID NO:19. The present application is also directed to nucleic acid molecules comprising, or alternatively, consisting of, a polynucleotide sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99%

identical to the polynucleotide sequence encoding the Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these nucleic acids and/or polynucleotide sequences are also encompassed by the invention, as are polypeptides comprising an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence described above, and polynucleotides that encode such polypeptides.

[0311] Similarly, deletions of C-terminal amino acid residues of the predicted extracellular domain of Neutrokin- $\alpha$ SV up to the leucine residue at position 79 of SEQ ID NO:19 may retain some functional activity, such as, for example, ligand binding, the ability to stimulate lymphocyte (e.g., B cell) proliferation, differentiation, and/or activation, modulation of cell replication, modulation of target cell activities and/or immunogenicity. Polypeptides having further C-terminal deletions including Leu-79 of SEQ ID NO:19 would not be expected to retain biological activities.

[0312] However, even if deletion of one or more amino acids from the C-terminus of a polypeptide results in modification or loss of one or more functional activities (e.g., biological activity) of the polypeptide, other functional activities may still be retained. Thus, the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete, mature or extracellular forms of the polypeptide generally will be retained when less than the majority of the residues of the complete, mature or extracellular forms of the polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of the predicted extracellular domain retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

[0313] Accordingly, the present invention further provides polypeptides having one or more residues from the carboxy terminus of the amino acid sequence of the predicted extracellular domain of Neutrokin- $\alpha$ SV shown in SEQ ID NO:19, up to the leucine residue at position 79 of SEQ ID NO:19, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides having the amino acid sequence of residues 73- $m^4$  of the amino acid sequence in SEQ ID NO:19, where  $m^4$  is any integer in the range of the amino acid position of amino acid residues 79-265 of the amino acid sequence in SEQ ID NO:19.

[0314] More in particular, in certain embodiments, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group consisting of residues Q-73 to L-265; Q-73 to K-264; Q-73 to L-263; Q-73 to A-262; Q-73 to G-261; Q-73 to F-260; Q-73 to F-259; Q-73 to T-258; Q-73 to V-257; Q-73 to D-256; Q-73 to G-255; Q-73 to D-254; Q-73 to L-253; Q-73 to S-252; Q-73 to I-251; Q-73 to Q-250; Q-73 to A-249; Q-73 to N-248; Q-73 to E-247; Q-73 to R-246; Q-73 to P-245; Q-73 to I-244; Q-73 to A-243; Q-73 to L-242; Q-73 to Q-241; Q-73 to L-240; Q-73 to E-239; Q-73 to D-238; Q-73 to G-237; Q-73 to E-236; Q-73 to E-235; Q-73 to L-234; Q-73 to K-233; Q-73 to A-232; Q-73 to I-231; Q-73 to G-230; Q-73 to A-229; Q-73 to S-228; Q-73 to Y-227; Q-73 to C-226; Q-73 to S-225; Q-73 to N-224; Q-73 to N-223; Q-73 to P-222; Q-73 to L-221; Q-73 to T-220; Q-73 to E-219; Q-73 to P-218; Q-73 to M-217; Q-73 to N-216; Q-73 to Q-215; Q-73 to I-214; Q-73 to C-213; Q-73 to R-212; Q-73 to F-211; Q-73 to L-210; Q-73 to T-209; Q-73 to V-208; Q-73 to L-207; Q-73 to S-206; Q-73 to L-205; Q-73 to E-204; Q-73 to D-203; Q-73 to G-202; Q-73 to F-201; Q-73 to V-200; Q-73 to H-199; Q-73 to V-198; Q-73 to K-197; Q-73 to K-196; Q-73 to R-195; Q-73 to Q-194; Q-73 to I-193; Q-73 to L-192; Q-73 to H-191; Q-73 to G-190; Q-73 to Q-7389; Q-73 to A-188; Q-73 to Y-187; Q-73 to T-186; Q-73 to K-185; Q-73 to D-184; Q-73 to T-183; Q-73 to Y-182; Q-73 to L-181; Q-73 to V-180; Q-73 to Q-179; Q-73 to G-178; Q-73 to Y-177; Q-73 to I-176; Q-73 to F-175; Q-73 to F-174; Q-73 to Y-173; Q-73 to G-172; Q-73 to T-171; Q-73 to E-170; Q-73 to K-169; Q-73 to V-168; Q-73 to L-167; Q-73 to I-166; Q-73 to K-165; Q-73 to N-164; Q-73 to E-163; Q-73 to K-162; Q-73 to E-161; Q-73 to E-160; Q-73 to L-159; Q-73 to A-158; Q-73 to S-157; Q-73 to G-156; Q-73 to R-155; Q-73 to K-154; Q-73 to F-153; Q-73 to S-152; Q-73 to L-151; Q-73 to L-150; Q-73 to W-149; Q-73 to P-148; Q-73 to V-147; Q-73 to F-146; Q-73 to T-145; Q-73 to Y-144; Q-73 to S-143; Q-73 to G-142; Q-73 to T-141; Q-73 to E-140; Q-73 to E-139; Q-73 to P-138; Q-73 to G-137; Q-73 to Q-136; Q-73 to V-135; Q-73 to A-134; Q-73 to R-133; Q-73 to K-132; Q-73 to N-131; Q-73 to R-130; Q-73 to S-129; Q-73 to N-128; Q-73 to Q-127; Q-73 to S-126; Q-73 to S-125; Q-73 to N-124; Q-73 to G-123; Q-73 to E-122; Q-73 to G-121; Q-73 to P-120; Q-73 to A-119; Q-73 to P-118; Q-73 to P-117; Q-73 to E-116; Q-73 to F-115; Q-73 to I-114; Q-73 to K-113; Q-73 to L-112; Q-73 to G-111; Q-73 to A-110; Q-73 to T-109; Q-73 to V-108; Q-73 to A-107; Q-73 to P-106; Q-73 to A-105;

Q-73 to E-104; Q-73 to E-103; Q-73 to L-102; Q-73 to G-101; Q-73 to A-100; Q-73 to K-99; Q-73 to P-98; Q-73 to A-97; Q-73 to G-96; Q-73 to A-95; Q-73 to G-94; Q-73 to A-93; Q-73 to P-92; Q-73 to L-91; Q-73 to K-90; Q-73 to E-89; Q-73 to A-88; Q-73 to H-87; Q-73 to H-86; Q-73 to G-85; Q-73 to Q-84; Q-73 to L-83; Q-73 to E-82; Q-73 to A-81; Q-73 to R-80; Q-73 to L-79; and Q-73 to S-78 of SEQ ID NO:19. The present application is also directed to nucleic acid molecules comprising, or alternatively, consisting of, a polynucleotide sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the polynucleotide sequence encoding the Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these nucleic acids and/or polynucleotide sequences are also encompassed by the invention, as are polypeptides comprising an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence described above, and polynucleotides that encode such polypeptides.

[0315] The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini of the predicted extracellular domain of Neutrokin- $\alpha$ SV, which may be described generally as having residues  $n^4$ - $m^4$  of SEQ ID NO:19 where  $n^4$  and  $m^4$  are integers as defined above.

[0316] In another embodiment, a nucleotide sequence encoding a polypeptide consisting of a portion of the extracellular domain of the Neutrokin- $\alpha$ SV amino acid sequence encoded by the cDNA clone contained in the deposit having ATCC Accession No. 203518, where this portion excludes from 1 to about 260 amino acids from the amino terminus of the extracellular domain of the amino acid sequence encoded by cDNA clone contained in the deposit having ATCC Accession No. 203518, or from 1 to about 187 amino acids from the carboxy terminus of the extracellular domain of the amino acid sequence encoded by cDNA clone contained in the deposit having ATCC Accession No. 203518, or any combination of the above amino terminal and carboxy terminal deletions, of the entire extracellular domain of the amino acid sequence encoded by the cDNA clone contained in the deposit having ATCC Accession No. 203518.

[0317] As mentioned above, even if deletion of one or more amino acids from the N-terminus of a polypeptide results in modification or loss of one or more functional activities (e.g., biological activity) of the polypeptide, other functional activities may still

be retained. Thus, the ability of a shortened Neutrokin- $\alpha$ SV mutein to induce and/or bind to antibodies which recognize the full-length or mature forms or the extracellular domain of the polypeptide generally will be retained when less than the majority of the residues of the full-length or mature or extracellular domain of the polypeptide are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a Neutrokin- $\alpha$ SV mutein with a large number of deleted N-terminal amino acid residues may retain functional (e.g., immunogenic) activities. In fact, peptides composed of as few as six Neutrokin- $\alpha$ SV amino acid residues may often evoke an immune response.

[0318] Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the predicted full-length amino acid sequence of the Neutrokin- $\alpha$ SV shown in SEQ ID NO:19, up to the glycine residue at position number 261 of the sequence shown SEQ ID NO:19 and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues n<sup>5</sup>-266 of the sequence shown in SEQ ID NO:19, where n<sup>5</sup> is an integer in the range of the amino acid position of amino acid residues 1 to 261 of the amino acid sequence in SEQ ID NO:19.

[0319] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group consisting of residues of D-2 to L-266; D-3 to L-266; S-4 to L-266; T-5 to L-266; E-6 to L-266; R-7 to L-266; E-8 to L-266; Q-9 to L-266; S-10 to L-266; R-11 to L-266; L-12 to L-266; T-13 to L-266; S-14 to L-266; C-15 to L-266; L-16 to L-266; K-17 to L-266; K-18 to L-266; R-19 to L-266; E-20 to L-266; E-21 to L-266; M-22 to L-266; K-23 to L-266; L-24 to L-266; K-25 to L-266; E-26 to L-266; C-27 to L-266; V-28 to L-266; S-29 to L-266; I-30 to L-266; L-31 to L-266; P-32 to L-266; R-33 to L-266; K-34 to L-266; E-35 to L-266; S-36 to L-266; P-37 to L-266; S-38 to L-266; V-39 to L-266; R-40 to L-266; S-41 to L-266; S-42 to L-266; K-43 to L-266; D-44 to L-266; G-45 to L-266; K-46 to L-266; L-47 to L-266; L-48 to L-266; A-49 to L-266; A-50 to L-266; T-51 to L-266; L-52 to L-266; L-53 to L-266; L-54 to L-266; A-55 to L-266; L-56 to L-266; L-57 to L-266; S-58 to L-266; C-59 to L-266; C-60 to L-266; L-61 to L-266; T-62 to



L-266; V-63 to L-266; V-64 to L-266; S-65 to L-266; F-66 to L-266; Y-67 to L-266; Q-68 to L-266; V-69 to L-266; A-70 to L-266; A-71 to L-266; L-72 to L-266; Q-73 to L-266; G-74 to L-266; D-75 to L-266; L-76 to L-266; A-77 to L-266; S-78 to L-266; L-79 to L-266; R-80 to L-266; A-81 to L-266; E-82 to L-266; L-83 to L-266; Q-84 to L-266; G-85 to L-266; H-86 to L-266; H-87 to L-266; A-88 to L-266; E-89 to L-266; K-90 to L-266; L-91 to L-266; P-92 to L-266; A-93 to L-266; G-94 to L-266; A-95 to L-266; G-96 to L-266; A-97 to L-266; P-98 to L-266; K-99 to L-266; A-100 to L-266; G-101 to L-266; L-102 to L-266; E-103 to L-266; E-104 to L-266; A-105 to L-266; P-106 to L-266; A-107 to L-266; V-108 to L-266; T-109 to L-266; A-110 to L-266; G-111 to L-266; L-112 to L-266; K-113 to L-266; I-114 to L-266; F-115 to L-266; E-116 to L-266; P-117 to L-266; P-118 to L-266; A-119 to L-266; P-120 to L-266; G-121 to L-266; E-122 to L-266; G-123 to L-266; N-124 to L-266; S-125 to L-266; S-126 to L-266; Q-127 to L-266; N-128 to L-266; S-129 to L-266; R-130 to L-266; N-131 to L-266; K-132 to L-266; R-133 to L-266; A-134 to L-266; V-135 to L-266; Q-136 to L-266; G-137 to L-266; P-138 to L-266; E-139 to L-266; E-140 to L-266; T-141 to L-266; G-142 to L-266; S-143 to L-266; Y-144 to L-266; T-145 to L-266; F-146 to L-266; V-147 to L-266; P-148 to L-266; W-149 to L-266; L-150 to L-266; L-151 to L-266; S-152 to L-266; F-153 to L-266; K-154 to L-266; R-155 to L-266; G-156 to L-266; S-157 to L-266; A-158 to L-266; L-159 to L-266; E-160 to L-266; E-161 to L-266; K-162 to L-266; E-163 to L-266; N-164 to L-266; K-165 to L-266; I-166 to L-266; L-167 to L-266; V-168 to L-266; K-169 to L-266; E-170 to L-266; T-171 to L-266; G-172 to L-266; Y-173 to L-266; F-174 to L-266; F-175 to L-266; I-176 to L-266; Y-177 to L-266; G-178 to L-266; Q-179 to L-266; V-180 to L-266; L-181 to L-266; Y-182 to L-266; T-183 to L-266; D-184 to L-266; K-185 to L-266; T-186 to L-266; Y-187 to L-266; A-188 to L-266; M-189 to L-266; G-190 to L-266; H-191 to L-266; L-192 to L-266; I-193 to L-266; Q-194 to L-266; R-195 to L-266; K-196 to L-266; K-197 to L-266; V-198 to L-266; H-199 to L-266; V-200 to L-266; F-201 to L-266; G-202 to L-266; D-203 to L-266; E-204 to L-266; L-205 to L-266; S-206 to L-266; L-207 to L-266; V-208 to L-266; T-209 to L-266; L-210 to L-266; F-211 to L-266; R-212 to L-266; C-213 to L-266; I-214 to L-266; Q-215 to L-266; N-216 to L-266; M-217 to L-266; P-218 to L-266; E-219 to L-266; T-220 to L-266; L-221 to L-266; P-222 to L-266; N-223 to L-266; N-224 to L-266; S-225 to L-266; C-226 to L-266; Y-227 to L-266; S-228 to L-266; A-229 to L-266; G-230 to L-266; I-231 to L-266; A-232 to L-266;

K-233 to L-266; L-234 to L-266; E-235 to L-266; E-236 to L-266; G-237 to L-266; D-238 to L-266; E-239 to L-266; L-240 to L-266; Q-241 to L-266; L-242 to L-266; A-243 to L-266; I-244 to L-266; P-245 to L-266; R-246 to L-266; E-247 to L-266; N-248 to L-266; A-249 to L-266; Q-250 to L-266; I-251 to L-266; S-252 to L-266; L-253 to L-266; D-254 to L-266; G-255 to L-266; D-256 to L-266; V-257 to L-266; T-258 to L-266; F-259 to L-266; F-260 to L-266; and G-261 to L-266 of SEQ ID NO:19. The present application is also directed to nucleic acid molecules comprising, or alternatively, consisting of, a polynucleotide sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the polynucleotide sequence encoding the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these nucleic acids and/or polynucleotide sequences are also encompassed by the invention, as are polypeptides comprising an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence described above, and polynucleotides that encode such polypeptides.

**[0320]** Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more functional activities (e.g., biological activities) of the protein, other functional activities may still be retained. Thus, the ability of a shortened Neutrokine-alphaSV mutein to induce and/or bind to antibodies which recognize the complete or mature form or the extracellular domain of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature form or the extracellular domain of the polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a Neutrokine-alphaSV mutein with a large number of deleted C-terminal amino acid residues may retain some functional (e.g., immunogenic) activities. In fact, peptides composed of as few as six Neutrokine-alphaSV amino acid residues may often evoke an immune response.

**[0321]** Accordingly, the present invention further provides in another embodiment, polypeptides having one or more residues deleted from the carboxy terminus of the amino acid sequence of the Neutrokine-alphaSV shown in SEQ ID NO:19, up to the glutamic

acid residue at position number 6, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues 1-m<sup>5</sup> of SEQ ID NO:19, where m<sup>5</sup> is an integer in the range of the amino acid position of amino acid residues 6 to 265 in the amino acid sequence of SEQ ID NO:19.

**[0322]** More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group consisting of residues M-1 to L-265; M-1 to K-264; M-1 to L-263; M-1 to A-262; M-1 to G-261; M-1 to F-260; M-1 to F-259; M-1 to T-258; M-1 to V-257; M-1 to D-256; M-1 to G-255; M-1 to D-254; M-1 to L-253; M-1 to S-252; M-1 to I-251; M-1 to Q-250; M-1 to A-249; M-1 to N-248; M-1 to E-247; M-1 to R-246; M-1 to P-245; M-1 to I-244; M-1 to A-243; M-1 to L-242; M-1 to Q-241; M-1 to L-240; M-1 to E-239; M-1 to D-238; M-1 to G-237; M-1 to E-236; M-1 to E-235; M-1 to L-234; M-1 to K-233; M-1 to A-232; M-1 to I-231; M-1 to G-230; M-1 to A-229; M-1 to S-228; M-1 to Y-227; M-1 to C-226; M-1 to S-225; M-1 to N-224; M-1 to N-223; M-1 to P-222; M-1 to L-221; M-1 to T-220; M-1 to E-219; M-1 to P-218; M-1 to M-217; M-1 to N-216; M-1 to Q-215; M-1 to I-214; M-1 to C-213; M-1 to R-212; M-1 to F-211; M-1 to L-210; M-1 to T-209; M-1 to V-208; M-1 to L-207; M-1 to S-206; M-1 to L-205; M-1 to E-204; M-1 to D-203; M-1 to G-202; M-1 to F-201; M-1 to V-200; M-1 to H-199; M-1 to V-198; M-1 to K-197; M-1 to K-196; M-1 to R-195; M-1 to Q-194; M-1 to I-193; M-1 to L-192; M-1 to H-191; M-1 to G-190; M-1 to M-189; M-1 to A-188; M-1 to Y-187; M-1 to T-186; M-1 to K-185; M-1 to D-184; M-1 to T-183; M-1 to Y-182; M-1 to L-181; M-1 to V-180; M-1 to Q-179; M-1 to G-178; M-1 to Y-177; M-1 to I-176; M-1 to F-175; M-1 to F-174; M-1 to Y-173; M-1 to G-172; M-1 to T-171; M-1 to E-170; M-1 to K-169; M-1 to V-168; M-1 to L-167; M-1 to I-166; M-1 to K-165; M-1 to N-164; M-1 to E-163; M-1 to K-162; M-1 to E-161; M-1 to E-160; M-1 to L-159; M-1 to A-158; M-1 to S-157; M-1 to G-156; M-1 to R-155; M-1 to K-154; M-1 to F-153; M-1 to S-152; M-1 to L-151; M-1 to L-150; M-1 to W-149; M-1 to P-148; M-1 to V-147; M-1 to F-146; M-1 to T-145; M-1 to Y-144; M-1 to S-143; M-1 to G-142; M-1 to T-141; M-1 to E-140; M-1 to E-139; M-1 to P-138; M-1 to G-137; M-1 to Q-136; M-1 to V-135; M-1 to A-134; M-1 to R-133; M-1 to K-132; M-1 to N-131; M-1 to R-130; M-1 to S-129; M-1 to N-128; M-1 to Q-127; M-1 to S-126; M-1 to S-125; M-1 to N-124; M-1 to G-123; M-1 to E-122; M-1 to G-121; M-1 to P-120; M-1 to A-119; M-1 to

P-118; M-1 to P-117; M-1 to E-116; M-1 to F-115; M-1 to I-114; M-1 to K-113; M-1 to L-112; M-1 to G-111; M-1 to A-110; M-1 to T-109; M-1 to V-108; M-1 to A-107; M-1 to P-106; M-1 to A-105; M-1 to E-104; M-1 to E-103; M-1 to L-102; M-1 to G-101; M-1 to A-100; M-1 to K-99; M-1 to P-98; M-1 to A-97; M-1 to G-96; M-1 to A-95; M-1 to G-94; M-1 to A-93; M-1 to P-92; M-1 to L-91; M-1 to K-90; M-1 to E-89; M-1 to A-88; M-1 to H-87; M-1 to H-86; M-1 to G-85; M-1 to Q-84; M-1 to L-83; M-1 to E-82; M-1 to A-81; M-1 to R-80; M-1 to L-79; M-1 to S-78; M-1 to A-77; M-1 to L-76; M-1 to D-75; M-1 to G-74; M-1 to Q-73; M-1 to L-72; M-1 to A-71; M-1 to A-70; M-1 to V-69; M-1 to Q-68; M-1 to Y-67; M-1 to F-66; M-1 to S-65; M-1 to V-64; M-1 to V-63; M-1 to T-62; M-1 to L-61; M-1 to C-60; M-1 to C-59; M-1 to S-58; M-1 to L-57; M-1 to L-56; M-1 to A-55; M-1 to L-54; M-1 to L-53; M-1 to L-52; M-1 to T-51; M-1 to A-50; M-1 to A-49; M-1 to L-48; M-1 to L-47; M-1 to K-46; M-1 to G-45; M-1 to D-44; M-1 to K-43; M-1 to S-42; M-1 to S-41; M-1 to R-40; M-1 to V-39; M-1 to S-38; M-1 to P-37; M-1 to S-36; M-1 to E-35; M-1 to K-34; M-1 to R-33; M-1 to P-32; M-1 to L-31; M-1 to I-30; M-1 to S-29; M-1 to V-28; M-1 to C-27; M-1 to E-26; M-1 to K-25; M-1 to L-24; M-1 to K-23; M-1 to M-22; M-1 to E-21; M-1 to E-20; M-1 to R-19; M-1 to K-18; M-1 to K-17; M-1 to L-16; M-1 to C-15; M-1 to S-14; M-1 to T-13; M-1 to L-12; M-1 to R-11; M-1 to S-10; M-1 to Q-9; M-1 to E-8; M-1 to R-7; and M-1 to E-6 of SEQ ID NO:19. The present application is also directed to nucleic acid molecules comprising, or alternatively, consisting of, a polynucleotide sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the polynucleotide sequence encoding the Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these nucleic acids and/or polynucleotide sequences are also encompassed by the invention, as are polypeptides comprising an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence described above, and polynucleotides that encode such polypeptides.

**[0323]** The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini of a Neutrokin- $\alpha$ SV polypeptide, which may be described generally as having residues  $n^5$ - $m^5$  of SEQ ID NO:19, where  $n^5$  and  $m^5$  are integers as defined above.

[0324] In additional embodiments, the present invention provides polypeptides comprising the amino acid sequence of residues 134-m<sup>6</sup> of SEQ ID NO:2, where m<sup>6</sup> is an integer from 140 to 285, corresponding to the position of the amino acid residue in SEQ ID NO:2. For example, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group consisting of residues A-134 to Leu-285; A-134 to L-284; A-134 to K-283; A-134 to L-282; A-134 to A-281; A-134 to G-280; A-134 to F-279; A-134 to F-278; A-134 to T-277; A-134 to V-276; A-134 to D-275; A-134 to G-274; A-134 to D-273; A-134 to L-272; A-134 to S-271; A-134 to I-270; A-134 to Q-269; A-134 to A-268; A-134 to N-267; A-134 to E-266; A-134 to R-265; A-134 to P-264; A-134 to I-263; A-134 to A-262; A-134 to L-261; A-134 to Q-260; A-134 to L-259; A-134 to E-258; A-134 to D-257; A-134 to G-256; A-134 to E-255; A-134 to E-254; A-134 to L-253; A-134 to K-252; A-134 to A-251; A-134 to I-250; A-134 to G-249; A-134 to A-248; A-134 to S-247; A-134 to Y-246; A-134 to C-245; A-134 to S-244; A-134 to N-243; A-134 to N-242; A-134 to P-241; A-134 to L-240; A-134 to T-239; A-134 to E-238; A-134 to P-237; A-134 to M-236; A-134 to N-235; A-134 to Q-234; A-134 to I-233; A-134 to C-232; A-134 to R-231; A-134 to F-230; A-134 to L-229; A-134 to T-228; A-134 to V-227; A-134 to L-226; A-134 to S-225; A-134 to L-224; A-134 to E-223; A-134 to D-222; A-134 to G-221; A-134 to F-220; A-134 to V-219; A-134 to H-218; A-134 to V-217; A-134 to K-216; A-134 to K-215; A-134 to R-214; A-134 to Q-213; A-134 to I-212; A-134 to L-211; A-134 to H-210; A-134 to G-209; A-134 to M-208; A-134 to A-207; A-134 to Y-206; A-134 to T-205; A-134 to K-204; A-134 to D-203; A-134 to T-202; A-134 to Y-201; A-134 to L-200; A-134 to V-199; A-134 to Q-198; A-134 to G-197; A-134 to Y-196; A-134 to I-195; A-134 to F-194; A-134 to F-193; A-134 to Y-192; A-134 to G-191; A-134 to T-190; A-134 to E-189; A-134 to K-188; A-134 to V-187; A-134 to L-186; A-134 to I-185; A-134 to K-184; A-134 to N-183; A-134 to E-182; A-134 to K-181; A-134 to E-180; A-134 to E-179; A-134 to L-178; A-134 to A-177; A-134 to S-176; A-134 to G-175; A-134 to R-174; A-134 to K-173; A-134 to F-172; A-134 to S-171; A-134 to L-170; A-134 to L-169; A-134 to W-168; A-134 to P-167; A-134 to V-166; A-134 to F-165; A-134 to T-164; A-134 to Y-163; A-134 to S-162; A-134 to G-161; A-134 to K-160; A-134 to Q-159; A-134 to I-158; A-134 to T-157; A-134 to P-156; A-134 to T-155; A-134 to E-154; A-134 to S-153; A-134 to

D-152; A-134 to A-151; A-134 to I-150; A-134 to L-149; A-134 to Q-148; A-134 to L-147; A-134 to C-146; A-134 to D-145; A-134 to Q-144; A-134 to T-143; A-134 to V-142; A-134 to T-141; and A-134 to E-140 of SEQ ID NO:2. The present application is also directed to nucleic acid molecules comprising, or alternatively, consisting of, a polynucleotide sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the polynucleotide sequence encoding the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these nucleic acids and/or polynucleotide sequences are also encompassed by the invention, as are polypeptides comprising an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence described above, and polynucleotides that encode such polypeptides.

**[0325]** Additional preferred polypeptide fragments of the invention comprise, or alternatively consist of, an amino acid sequence selected from the group consisting of residues: M-1 to C-15; D-2 to L-16; D-3 to K-17; S-4 to K-18; T-5 to R-19; E-6 to E-20; R-7 to E-21; E-8 to M-22; Q-9 to K-23; S-10 to L-24; R-11 to K-25; L-12 to E-26; T-13 to C-27; S-14 to V-28; C-15 to S-29; L-16 to I-30; K-17 to L-31; K-18 to P-32; R-19 to R-33; E-20 to K-34; E-21 to E-35; M-22 to S-36; K-23 to P-37; L-24 to S-38; K-25 to V-39; E-26 to R-40; C-27 to S-41; V-28 to S-42; S-29 to K-43; I-30 to D-44; L-31 to G-45; P-32 to K-46; R-33 to L-47; K-34 to L-48; E-35 to A-49; S-36 to A-50; P-37 to T-51; S-38 to L-52; V-39 to L-53; R-40 to L-54; S-41 to A-55; S-42 to L-56; K-43 to L-57; D-44 to S-58; G-45 to C-59; K-46 to C-60; L-47 to L-61; L-48 to T-62; A-49 to V-63; A-50 to V-64; T-51 to S-65; L-52 to F-66; L-53 to Y-67; L-54 to Q-68; A-55 to V-69; L-56 to A-70; L-57 to A-71; S-58 to L-72; C-59 to Q-73; C-60 to G-74; L-61 to D-75; T-62 to L-76; V-63 to A-77; V-64 to S-78; S-65 to L-79; F-66 to R-80; Y-67 to A-81; Q-68 to E-82; V-69 to L-83; A-70 to Q-84; A-71 to G-85; L-72 to H-86; Q-73 to H-87; G-74 to A-88; D-75 to E-89; L-76 to K-90; A-77 to L-91; S-78 to P-92; L-79 to A-93; R-80 to G-94; A-81 to A-95; E-82 to G-96; L-83 to A-97; Q-84 to P-98; G-85 to K-99; H-86 to A-100; H-87 to G-101; A-88 to L-102; E-89 to E-103; K-90 to E-104; L-91 to A-105; P-92 to P-106; A-93 to A-107; G-94 to V-108; A-95 to T-109; G-96 to A-110; A-97 to G-111; P-98 to L-112; K-99 to K-113; A-100 to I-114; G-101 to F-115; L-102 to E-116; E-103 to P-117; E-104 to P-118; A-105 to A-119; P-106 to P-120; A-107 to G-121; V-108 to E-122; T-109 to G-123;

A-110 to N-124; G-111 to S-125; L-112 to S-126; K-113 to Q-127; I-114 to N-128; F-115 to S-129; E-116 to R-130; P-117 to N-131; P-118 to K-132; A-119 to R-133; P-120 to A-134; G-121 to V-135; E-122 to Q-136; G-123 to G-137; N-124 to P-138; S-125 to E-139; S-126 to E-140; Q-127 to T-141; N-128 to V-142; S-129 to T-143; R-130 to Q-144; N-131 to D-145; K-132 to C-146; R-133 to L-147; A-134 to Q-148; V-135 to L-149; Q-136 to I-150; G-137 to A-151; P-138 to D-152; E-139 to S-153; E-140 to E-154; T-141 to T-155; V-142 to P-156; T-143 to T-157; Q-144 to I-158; D-145 to Q-159; C-146 to K-160; L-147 to G-161; Q-148 to S-162; L-149 to Y-163; I-150 to T-164; A-151 to F-165; D-152 to V-166; S-153 to P-167; E-154 to W-168; T-155 to L-169; P-156 to L-170; T-157 to S-171; I-158 to F-172; Q-159 to K-173; K-160 to R-174; G-161 to G-175; S-162 to S-176; Y-163 to A-177; T-164 to L-178; F-165 to E-179; V-166 to E-180; P-167 to K-181; W-168 to E-182; L-169 to N-183; L-170 to K-184; S-171 to I-185; F-172 to L-186; K-173 to V-187; R-174 to K-188; G-175 to E-189; S-176 to T-190; A-177 to G-191; L-178 to Y-192; E-179 to F-193; E-180 to F-194; K-181 to I-195; E-182 to Y-196; N-183 to G-197; K-184 to Q-198; I-185 to V-199; L-186 to L-200; V-187 to Y-201; K-188 to T-202; E-189 to D-203; T-190 to K-204; G-191 to T-205; Y-192 to Y-206; F-193 to A-207; F-194 to M-208; I-195 to G-209; Y-196 to H-210; G-197 to L-211; Q-198 to I-212; V-199 to Q-213; L-200 to R-214; Y-201 to K-215; T-202 to K-216; D-203 to V-217; K-204 to H-218; T-205 to V-219; Y-206 to F-220; A-207 to G-221; M-208 to D-222; G-209 to E-223; H-210 to L-224; L-211 to S-225; I-212 to L-226; Q-213 to V-227; R-214 to T-228; K-215 to L-229; K-216 to F-230; V-217 to R-231; H-218 to C-232; V-219 to I-233; F-220 to Q-234; G-221 to N-235; D-222 to M-236; E-223 to P-237; L-224 to E-238; S-225 to T-239; L-226 to L-240; V-227 to P-241; T-228 to N-242; L-229 to N-243; F-230 to S-244; R-231 to C-245; C-232 to Y-246; I-233 to S-247; Q-234 to A-248; N-235 to G-249; M-236 to I-250; P-237 to A-251; E-238 to K-252; T-239 to L-253; L-240 to E-254; P-241 to E-255; N-242 to G-256; N-243 to D-257; S-244 to E-258; C-245 to L-259; Y-246 to Q-260; S-247 to L-261; A-248 to A-262; G-249 to I-263; I-250 to P-264; A-251 to R-265; K-252 to E-266; L-253 to N-267; E-254 to A-268; E-255 to Q-269; G-256 to I-270; D-257 to S-271; E-258 to L-272; L-259 to D-273; Q-260 to G-274; L-261 to D-275; A-262 to V-276; I-263 to T-277; P-264 to F-278; R-265 to F-279; E-266 to G-280; N-267 to A-281; A-268 to L-282; Q-269 to K-283; I-270 to L-284; and S-271 to L-285 of SEQ ID NO:2. Preferably, these polypeptide fragments have one or more functional activities (e.g.,

biological activity, antigenicity, and immunogenicity) of Neutrokin-alpha and/or Neutrokin-alpha SV polypeptides of the invention and may be used, for example, to generate or screen for antibodies, as described further below. The present invention is also directed to polypeptides comprising, or alternatively, consisting of, an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence described above. The present invention also encompasses the above amino acid sequences fused to a heterologous amino acid sequence as described herein. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0326] Additional preferred polypeptide fragments of the invention comprise, or alternatively consist of, an amino acid sequence selected from the group consisting of residues: M-1 to C-15; D-2 to L-16; D-3 to K-17; S-4 to K-18; T-5 to R-19; E-6 to E-20; R-7 to E-21; E-8 to M-22; Q-9 to K-23; S-10 to L-24; R-11 to K-25; L-12 to E-26; T-13 to C-27; S-14 to V-28; C-15 to S-29; L-16 to I-30; K-17 to L-31; K-18 to P-32; R-19 to R-33; E-20 to K-34; E-21 to E-35; M-22 to S-36; K-23 to P-37; L-24 to S-38; K-25 to V-39; E-26 to R-40; C-27 to S-41; V-28 to S-42; S-29 to K-43; I-30 to D-44; L-31 to G-45; P-32 to K-46; R-33 to L-47; K-34 to L-48; E-35 to A-49; S-36 to A-50; P-37 to T-51; S-38 to L-52; V-39 to L-53; R-40 to L-54; S-41 to A-55; S-42 to L-56; K-43 to L-57; D-44 to S-58; G-45 to C-59; K-46 to C-60; L-47 to L-61; L-48 to T-62; A-49 to V-63; A-50 to V-64; T-51 to S-65; L-52 to F-66; L-53 to Y-67; L-54 to Q-68; A-55 to V-69; L-56 to A-70; L-57 to A-71; S-58 to L-72; C-59 to Q-73; C-60 to G-74; L-61 to D-75; T-62 to L-76; V-63 to A-77; V-64 to S-78; S-65 to L-79; F-66 to R-80; Y-67 to A-81; Q-68 to E-82; V-69 to L-83; A-70 to Q-84; A-71 to G-85; L-72 to H-86; Q-73 to H-87; G-74 to A-88; D-75 to E-89; L-76 to K-90; A-77 to L-91; S-78 to P-92; L-79 to A-93; R-80 to G-94; A-81 to A-95; E-82 to G-96; L-83 to A-97; Q-84 to P-98; G-85 to K-99; H-86 to A-100; H-87 to G-101; A-88 to L-102; E-89 to E-103; K-90 to E-104; L-91 to A-105; P-92 to P-106; A-93 to A-107; G-94 to V-108; A-95 to T-109; G-96 to A-110; A-97 to G-111; P-98 to L-112; K-99 to K-113; A-100 to I-114; G-101 to F-115; L-102 to E-116; E-103 to P-117; E-104 to P-118; A-105 to A-119; P-106 to P-120; A-107 to G-121; V-108 to E-122; T-109 to G-123; A-110 to N-124; G-111 to S-125; L-112 to S-126; K-113 to Q-127; I-114 to N-128; F-115 to S-129; E-116 to R-130; P-117 to N-131; P-118 to K-132; A-119 to R-133; P-120 to A-134; G-121 to V-135; E-122 to Q-136; G-123 to G-137; N-124 to P-138; S-125 to E-139; S-126 to E-140; Q-127 to T-141; N-128 to G-142; S-129 to S-143; R-130 to Y-144; N-131



to T-145; K-132 to F-146; R-133 to V-147; A-134 to P-148; V-135 to W-149; Q-136 to L-150; G-137 to L-151; P-138 to S-152; E-139 to F-153; E-140 to K-154; T-141 to R-155; G-142 to G-156; S-143 to S-157; Y-144 to A-158; T-145 to L-159; F-146 to E-160; V-147 to E-161; P-148 to K-162; W-149 to E-163; L-150 to N-164; L-151 to K-165; S-152 to I-166; F-153 to L-167; K-154 to V-168; R-155 to K-169; G-156 to E-170; S-157 to T-171; A-158 to G-172; L-159 to Y-173; E-160 to F-174; E-161 to F-175; K-162 to I-176; E-163 to Y-177; N-164 to G-178; K-165 to Q-179; I-166 to V-180; L-167 to L-181; V-168 to Y-182; K-169 to T-183; E-170 to D-184; T-171 to K-185; G-172 to T-186; Y-173 to Y-187; F-174 to A-188; F-175 to M-189; I-176 to G-190; Y-177 to H-191; G-178 to L-192; Q-179 to I-193; V-180 to Q-194; L-181 to R-195; Y-182 to K-196; T-183 to K-197; D-184 to V-198; K-185 to H-199; T-186 to V-200; Y-187 to F-201; A-188 to G-202; M-189 to D-203; G-190 to E-204; H-191 to L-205; L-192 to S-206; I-193 to L-207; Q-194 to V-208; R-195 to T-209; K-196 to L-210; K-197 to F-211; V-198 to R-212; H-199 to C-213; V-200 to I-214; F-201 to Q-215; G-202 to N-216; D-203 to M-217; E-204 to P-218; L-205 to E-219; S-206 to T-220; L-207 to L-221; V-208 to P-222; T-209 to N-223; L-210 to N-224; F-211 to S-225; R-212 to C-226; C-213 to Y-227; I-214 to S-228; Q-215 to A-229; N-216 to G-230; M-217 to I-231; P-218 to A-232; E-219 to K-233; T-220 to L-234; L-221 to E-235; P-222 to E-236; N-223 to G-237; N-224 to D-238; S-225 to E-239; C-226 to L-240; Y-227 to Q-241; S-228 to L-242; A-229 to A-243; G-230 to I-244; I-231 to P-245; A-232 to R-246; K-233 to E-247; L-234 to N-248; E-235 to A-249; E-236 to Q-250; G-237 to I-251; D-238 to S-252; E-239 to L-253; L-240 to D-254; Q-241 to G-255; L-242 to D-256; A-243 to V-257; I-244 to T-258; P-245 to F-259; R-246 to F-260; E-247 to G-261; N-248 to A-262; A-249 to L-263; Q-250 to K-264; I-251 to L-265; and S-252 to L-266 of SEQ ID NO:19. Preferably, these polypeptide fragments have one or more functional activities (e.g., biological activity, antigenicity, and immunogenicity) of Neutrokin-alpha and/or Neutrokin-alpha SV polypeptides of the invention and may be used, for example, to generate or screen for antibodies, as described further below. The present invention is also directed to polypeptides comprising, or alternatively, consisting of, an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence described above. The present invention also encompasses the above amino acid sequences fused to a heterologous amino acid sequence as described herein. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0327] Additional preferred polypeptide fragments of the invention comprise, or alternatively consist of, an amino acid sequence selected from the group consisting of residues: M-1 to F-15; D-2 to C-16; E-3 to S-17; S-4 to E-18; A-5 to K-19; K-6 to G-20; T-7 to E-21; L-8 to D-22; P-9 to M-23; P-10 to K-24; P-11 to V-25; C-12 to G-26; L-13 to Y-27; C-14 to D-28; F-15 to P-29; C-16 to I-30; S-17 to T-31; E-18 to P-32; K-19 to Q-33; G-20 to K-34; E-21 to E-35; D-22 to E-36; M-23 to G-37; K-24 to A-38; V-25 to W-39; G-26 to F-40; Y-27 to G-41; D-28 to I-42; P-29 to C-43; I-30 to R-44; T-31 to D-45; P-32 to G-46; Q-33 to R-47; K-34 to L-48; E-35 to L-49; E-36 to A-50; G-37 to A-51; A-38 to T-52; W-39 to L-53; F-40 to L-54; G-41 to L-55; I-42 to A-56; C-43 to L-57; R-44 to L-58; D-45 to S-59; G-46 to S-60; R-47 to S-61; L-48 to F-62; L-49 to T-63; A-50 to A-64; A-51 to M-65; T-52 to S-66; L-53 to L-67; L-54 to Y-68; L-55 to Q-69; A-56 to L-70; L-57 to A-71; L-58 to A-72; S-59 to L-73; S-60 to Q-74; S-61 to A-75; F-62 to D-76; T-63 to L-77; A-64 to M-78; M-65 to N-79; S-66 to L-80; L-67 to R-81; Y-68 to M-82; Q-69 to E-83; L-70 to L-84; A-71 to Q-85; A-72 to S-86; L-73 to Y-87; Q-74 to R-88; A-75 to G-89; D-76 to S-90; L-77 to A-91; M-78 to T-92; N-79 to P-93; L-80 to A-94; R-81 to A-95; M-82 to A-96; E-83 to G-97; L-84 to A-98; Q-85 to P-99; S-86 to E-100; Y-87 to L-101; R-88 to T-102; G-89 to A-103; S-90 to G-104; A-91 to V-105; T-92 to K-106; P-93 to L-107; A-94 to L-108; A-95 to T-109; A-96 to P-110; G-97 to A-111; A-98 to A-112; P-99 to P-113; E-100 to R-114; L-101 to P-115; T-102 to H-116; A-103 to N-117; G-104 to S-118; V-105 to S-119; K-106 to R-120; L-107 to G-121; L-108 to H-122; T-109 to R-123; P-110 to N-124; A-111 to R-125; A-112 to R-126; P-113 to A-127; R-114 to F-128; P-115 to Q-129; H-116 to G-130; N-117 to P-131; S-118 to E-132; S-119 to E-133; R-120 to T-134; G-121 to E-135; H-122 to Q-136; R-123 to D-137; N-124 to V-138; R-125 to D-139; R-126 to L-140; A-127 to S-141; F-128 to A-142; Q-129 to P-143; G-130 to P-144; P-131 to A-145; E-132 to P-146; E-133 to C-147; T-134 to L-148; E-135 to P-149; Q-136 to G-150; D-137 to C-151; V-138 to R-152; D-139 to H-153; L-140 to S-154; S-141 to Q-155; A-142 to H-156; P-143 to D-157; P-144 to D-158; A-145 to N-159; P-146 to G-160; C-147 to M-161; L-148 to N-162; P-149 to L-163; G-150 to R-164; C-151 to N-165; R-152 to I-166; H-153 to I-167; S-154 to Q-168; Q-155 to D-169; H-156 to C-170; D-157 to L-171; D-158 to Q-172; N-159 to L-173; G-160 to I-174; M-161 to A-175; N-162 to D-176; L-163 to S-177; R-164 to D-178; N-165 to T-179; I-166 to P-180; I-167 to A-181; Q-168 to L-182; D-169 to E-183; C-170 to E-184; L-171 to K-185; Q-172 to E-

186; L-173 to N-187; I-174 to K-188; A-175 to I-189; D-176 to V-190; S-177 to V-191; D-178 to R-192; T-179 to Q-193; P-180 to T-194; A-181 to G-195; L-182 to Y-196; E-183 to F-197; E-184 to F-198; K-185 to I-199; E-186 to Y-200; N-187 to S-201; K-188 to Q-202; I-189 to V-203; V-190 to L-204; V-191 to Y-205; R-192 to T-206; Q-193 to D-207; T-194 to P-208; G-195 to I-209; Y-196 to F-210; F-197 to A-211; F-198 to M-212; I-199 to G-213; Y-200 to H-214; S-201 to V-215; Q-202 to I-216; V-203 to Q-217; L-204 to R-218; Y-205 to K-219; T-206 to K-220; D-207 to V-221; P-208 to H-222; I-209 to V-223; F-210 to F-224; A-211 to G-225; M-212 to D-226; G-213 to E-227; H-214 to L-228; V-215 to S-229; I-216 to L-230; Q-217 to V-231; R-218 to T-232; K-219 to L-233; K-220 to F-234; V-221 to R-235; H-222 to C-236; V-223 to I-237; F-224 to Q-238; G-225 to N-239; D-226 to M-240; E-227 to P-241; L-228 to K-242; S-229 to T-243; L-230 to L-244; V-231 to P-245; T-232 to N-246; L-233 to N-247; F-234 to S-248; R-235 to C-249; C-236 to Y-250; I-237 to S-251; Q-238 to A-252; N-239 to G-253; M-240 to I-254; P-241 to A-255; K-242 to R-256; T-243 to L-257; L-244 to E-258; P-245 to E-259; N-246 to G-260; N-247 to D-261; S-248 to E-262; C-249 to I-263; Y-250 to Q-264; S-251 to L-265; A-252 to A-266; G-253 to I-267; I-254 to P-268; A-255 to R-269; R-256 to E-270; L-257 to N-271; E-258 to A-272; E-259 to Q-273; G-260 to I-274; D-261 to S-275; E-262 to R-276; I-263 to N-277; Q-264 to G-278; L-265 to D-279; A-266 to D-280; I-267 to T-281; P-268 to F-282; R-269 to F-283; E-270 to G-284; N-271 to A-285; A-272 to L-286; Q-273 to K-287; I-274 to L-288; and S-275 to L-289 of SEQ ID NO:38. Preferably, these polypeptide fragments have one or more functional activities (e.g., biological activity, antigenicity, and immunogenicity) of Neutrokin-alpha and/or Neutrokin-alpha SV polypeptides of the invention and may be used, for example, to generate or screen for antibodies, as described further below. The present invention is also directed to polypeptides comprising, or alternatively, consisting of, an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence described above. The present invention also encompasses the above amino acid sequences fused to a heterologous amino acid sequence as described herein. Polynucleotides encoding these polypeptides are also encompassed by the invention.

**[0328]** It will be recognized by one of ordinary skill in the art that some amino acid sequences of the Neutrokin-alpha and Neutrokin-alphaSV polypeptides can be varied without significant effect of the structure or function of the polypeptide. If such

differences in sequence are contemplated, it should be remembered that there will be critical areas on the polypeptide which determine activity.

[0329] Thus, the invention further includes variations of the Neutrokin- $\alpha$  polypeptide which show Neutrokin- $\alpha$  polypeptide functional activity (e.g., biological activity) or which include regions of Neutrokin- $\alpha$  polypeptide such as the polypeptide fragments described herein. The invention also includes variations of the Neutrokin- $\alpha$ SV polypeptide which show Neutrokin- $\alpha$ SV polypeptide functional activity (e.g., biological activity) or which include regions of Neutrokin- $\alpha$ SV polypeptide such as the polypeptide fragments described herein. Such mutants include deletions, insertions, inversions, repeats, and type substitutions selected according to general rules known in the art so as to have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, J. U. et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science* 247:1306-1310 (1990), wherein the authors indicate that there are two main approaches for studying the tolerance of an amino acid sequence to change. The first method relies on the process of evolution, in which mutations are either accepted or rejected by natural selection. The second approach uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene and selections or screens to identify sequences that maintain functionality.

[0330] As the authors state, these studies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at a certain position of the protein. For example, most buried amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Other such phenotypically silent substitutions are described in Bowie, J. U. *et al.*, *supra*, and the references cited therein. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg and replacements among the aromatic residues Phe, Tyr.

[0331] Thus, the fragment, derivative or analog of the polypeptide of Figures 1A and 1B (SEQ ID NO:2), or that encoded by the deposited cDNA plasmid, may be (i) one in

which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the extracellular domain of the polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the extracellular domain of the polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the extracellular domain of the polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

**[0332]** Furthermore, the fragment, derivative or analog of the polypeptide of Figures 5A and 5B (SEQ ID NO:19), or that encoded by the deposited cDNA plasmid, may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the extracellular domain of the polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the extracellular domain of the polypeptide, such as, a soluble biologically active fragment of another TNF ligand family member (e.g., CD40 Ligand), an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the extracellular domain of the polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

**[0333]** Thus, the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides of the present invention may include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation. As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table II).

TABLE II. Conservative Amino Acid Substitutions.

Aromatic	Phenylalanine Tryptophan Tyrosine
Hydrophobic	Leucine Isoleucine Valine
Polar	Glutamine Asparagine
Basic	Arginine Lysine Histidine
Acidic	Aspartic Acid Glutamic Acid
Small	Alanine Serine Threonine Methionine Glycine

[0334] In one embodiment of the invention, polypeptide comprises, or alternatively consists of, the amino acid sequence of a Neutrokin- $\alpha$  or Neutrokin- $\alpha$ SV polypeptide having an amino acid sequence which contains at least one conservative amino acid substitution, but not more than 50 conservative amino acid substitutions, even more preferably, not more than 40 conservative amino acid substitutions, still more preferably, not more than 30 conservative amino acid substitutions, and still even more preferably, not more than 20 conservative amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a peptide or polypeptide to have an amino acid sequence which comprises the amino acid sequence of a Neutrokin- $\alpha$  polypeptide, which contains at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 conservative amino acid substitutions.

[0335] For example, site directed changes at the amino acid level of Neutrokin- $\alpha$  can be made by replacing a particular amino acid with a conservative substitution. Preferred conservative substitution mutations of the Neutrokin- $\alpha$  amino acid sequence provided in SEQ ID NO:2 include: M1 replaced with A, G, I, L, S, T, or V; D2

replaced with E; D3 replaced with E; S4 replaced with A, G, I, L, T, M, or V; T5 replaced with A, G, I, L, S, M, or V; E6 replaced with D; R7 replaced with H, or K; E8 replaced with D; Q9 replaced with N; S10 replaced with A, G, I, L, T, M, or V; R11 replaced with H, or K; L12 replaced with A, G, I, S, T, M, or V; T13 replaced with A, G, I, L, S, M, or V; S14 replaced with A, G, I, L, T, M, or V; L16 replaced with A, G, I, S, T, M, or V; K17 replaced with H, or R; K18 replaced with H, or R; R19 replaced with H, or K; E20 replaced with D; E21 replaced with D; M22 replaced with A, G, I, L, S, T, or V; K23 replaced with H, or R; L24 replaced with A, G, I, S, T, M, or V; K25 replaced with H, or R; E26 replaced with D; V28 replaced with A, G, I, L, S, T, or M; S29 replaced with A, G, I, L, T, M, or V; I30 replaced with A, G, I, L, S, T, M, or V; L31 replaced with A, G, I, S, T, M, or V; R33 replaced with H, or K; K34 replaced with H, or R; E35 replaced with D; S36 replaced with A, G, I, L, T, M, or V; S38 replaced with A, G, I, L, T, M, or V; V39 replaced with A, G, I, L, S, T, or M; R40 replaced with H, or K; S41 replaced with A, G, I, L, T, M, or V; S42 replaced with A, G, I, L, T, M, or V; K43 replaced with H, or R; D44 replaced with E; G45 replaced with A, I, L, S, T, M, or V; K46 replaced with H, or R; L47 replaced with A, G, I, S, T, M, or V; L48 replaced with A, G, I, S, T, M, or V; A49 replaced with G, I, L, S, T, M, or V; A50 replaced with G, I, L, S, T, M, or V; T51 replaced with A, G, I, L, S, M, or V; L52 replaced with A, G, I, S, T, M, or V; L53 replaced with A, G, I, S, T, M, or V; L54 replaced with A, G, I, S, T, M, or V; A55 replaced with G, I, L, S, T, M, or V; L56 replaced with A, G, I, S, T, M, or V; L57 replaced with A, G, I, S, T, M, or V; S58 replaced with A, G, I, L, T, M, or V; L61 replaced with A, G, I, S, T, M, or V; T62 replaced with A, G, I, L, S, M, or V; V63 replaced with A, G, I, L, S, T, or M; V64 replaced with A, G, I, L, S, T, or M; S65 replaced with A, G, I, L, T, M, or V; F66 replaced with W, or Y; Y67 replaced with F, or W; Q68 replaced with N; V69 replaced with A, G, I, L, S, T, or M; A70 replaced with G, I, L, S, T, M, or V; A71 replaced with G, I, L, S, T, M, or V; L72 replaced with A, G, I, S, T, M, or V; Q73 replaced with N; G74 replaced with A, I, L, S, T, M, or V; D75 replaced with E; L76 replaced with A, G, I, S, T, M, or V; A77 replaced with G, I, L, S, T, M, or V; S78 replaced with A, G, I, L, T, M, or V; L79 replaced with A, G, I, S, T, M, or V; R80 replaced with H, or K; A81 replaced with G, I, L, S, T, M, or V; E82 replaced with D; L83 replaced with A, G, I, S, T, M, or V; Q84 replaced with N; G85 replaced with A, I, L, S, T, M, or V; H86 replaced with K, or R; H87 replaced with K, or R; A88 replaced with G,

I, L, S, T, M, or V; E89 replaced with D; K90 replaced with H, or R; L91 replaced with A, G, I, S, T, M, or V; A93 replaced with G, I, L, S, T, M, or V; G94 replaced with A, I, L, S, T, M, or V; A95 replaced with G, I, L, S, T, M, or V; G96 replaced with A, I, L, S, T, M, or V; A97 replaced with G, I, L, S, T, M, or V; K99 replaced with H, or R; A100 replaced with G, I, L, S, T, M, or V; G101 replaced with A, I, L, S, T, M, or V; L102 replaced with A, G, I, S, T, M, or V; E103 replaced with D; E104 replaced with D; A105 replaced with G, I, L, S, T, M, or V; A107 replaced with G, I, L, S, T, M, or V; V108 replaced with A, G, I, L, S, T, or M; T109 replaced with A, G, I, L, S, M, or V; A110 replaced with G, I, L, S, T, M, or V; G111 replaced with A, I, L, S, T, M, or V; L112 replaced with A, G, I, S, T, M, or V; K113 replaced with H, or R; I114 replaced with A, G, L, S, T, M, or V; F115 replaced with W, or Y; E116 replaced with D; A119 replaced with G, I, L, S, T, M, or V; G121 replaced with A, I, L, S, T, M, or V; E122 replaced with D; G123 replaced with A, I, L, S, T, M, or V; N124 replaced with Q; S125 replaced with A, G, I, L, T, M, or V; S126 replaced with A, G, I, L, T, M, or V; Q127 replaced with N; N128 replaced with Q; S129 replaced with A, G, I, L, T, M, or V; R130 replaced with H, or K; N131 replaced with Q; K132 replaced with H, or R; R133 replaced with H, or K; A134 replaced with G, I, L, S, T, M, or V; V135 replaced with A, G, I, L, S, T, or M; Q136 replaced with N; G137 replaced with A, I, L, S, T, M, or V; E139 replaced with D; E140 replaced with D; T141 replaced with A, G, I, L, S, M, or V; V142 replaced with A, G, I, L, S, T, or M; T143 replaced with A, G, I, L, S, M, or V; Q144 replaced with N; D145 replaced with E; L147 replaced with A, G, I, S, T, M, or V; Q148 replaced with N; L149 replaced with A, G, I, S, T, M, or V; I150 replaced with A, G, L, S, T, M, or V; A151 replaced with G, I, L, S, T, M, or V; D152 replaced with E; S153 replaced with A, G, I, L, T, M, or V; E154 replaced with D; T155 replaced with A, G, I, L, S, M, or V; T157 replaced with A, G, I, L, S, M, or V; I158 replaced with A, G, L, S, T, M, or V; Q159 replaced with N; K160 replaced with H, or R; G161 replaced with A, I, L, S, T, M, or V; S162 replaced with A, G, I, L, T, M, or V; Y163 replaced with F, or W; T164 replaced with A, G, I, L, S, M, or V; F165 replaced with W, or Y; V166 replaced with A, G, I, L, S, T, or M; W168 replaced with F, or Y; L169 replaced with A, G, I, S, T, M, or V; L170 replaced with A, G, I, S, T, M, or V; S171 replaced with A, G, I, L, T, M, or V; F172 replaced with W, or Y; K173 replaced with H, or R; R174 replaced with H, or K; G175 replaced with A, I, L, S, T, M, or V; S176 replaced with A, G, I, L, T, M, or V; A177 replaced with G, I, L, S, T, M, or



V; L178 replaced with A, G, I, S, T, M, or V; E179 replaced with D; E180 replaced with D; K181 replaced with H, or R; E182 replaced with D; N183 replaced with Q; K184 replaced with H, or R; I185 replaced with A, G, L, S, T, M, or V; L186 replaced with A, G, I, S, T, M, or V; V187 replaced with A, G, I, L, S, T, or M; K188 replaced with H, or R; E189 replaced with D; T190 replaced with A, G, I, L, S, M, or V; G191 replaced with A, I, L, S, T, M, or V; Y192 replaced with F, or W; F193 replaced with W, or Y; F194 replaced with W, or Y; I195 replaced with A, G, L, S, T, M, or V; Y196 replaced with F, or W; G197 replaced with A, I, L, S, T, M, or V; Q198 replaced with N; V199 replaced with A, G, I, L, S, T, or M; L200 replaced with A, G, I, S, T, M, or V; Y201 replaced with F, or W; T202 replaced with A, G, I, L, S, M, or V; D203 replaced with E; K204 replaced with H, or R; T205 replaced with A, G, I, L, S, M, or V; Y206 replaced with F, or W; A207 replaced with G, I, L, S, T, M, or V; M208 replaced with A, G, I, L, S, T, or V; G209 replaced with A, I, L, S, T, M, or V; H210 replaced with K, or R; L211 replaced with A, G, I, S, T, M, or V; I212 replaced with A, G, L, S, T, M, or V; Q213 replaced with N; R214 replaced with H, or K; K215 replaced with H, or R; K216 replaced with H, or R; V217 replaced with A, G, I, L, S, T, or M; H218 replaced with K, or R; V219 replaced with A, G, I, L, S, T, or M; F220 replaced with W, or Y; G221 replaced with A, I, L, S, T, M, or V; D222 replaced with E; E223 replaced with D; L224 replaced with A, G, I, S, T, M, or V; S225 replaced with A, G, I, L, T, M, or V; L226 replaced with A, G, I, S, T, M, or V; V227 replaced with A, G, I, L, S, T, or M; T228 replaced with A, G, I, L, S, M, or V; L229 replaced with A, G, I, S, T, M, or V; F230 replaced with W, or Y; R231 replaced with H, or K; I233 replaced with A, G, L, S, T, M, or V; Q234 replaced with N; N235 replaced with Q; M236 replaced with A, G, I, L, S, T, or V; E238 replaced with D; T239 replaced with A, G, I, L, S, M, or V; L240 replaced with A, G, I, S, T, M, or V; N242 replaced with Q; N243 replaced with Q; S244 replaced with A, G, I, L, T, M, or V; Y246 replaced with F, or W; S247 replaced with A, G, I, L, T, M, or V; A248 replaced with G, I, L, S, T, M, or V; G249 replaced with A, I, L, S, T, M, or V; I250 replaced with A, G, L, S, T, M, or V; A251 replaced with G, I, L, S, T, M, or V; K252 replaced with H, or R; L253 replaced with A, G, I, S, T, M, or V; E254 replaced with D; E255 replaced with D; G256 replaced with A, I, L, S, T, M, or V; D257 replaced with E; E258 replaced with D; L259 replaced with A, G, I, S, T, M, or V; Q260 replaced with N; L261 replaced with A, G, I, S, T, M, or V; A262 replaced with G, I, L, S, T, M, or V; I263 replaced with A, G, L,

S, T, M, or V; R265 replaced with H, or K; E266 replaced with D; N267 replaced with Q; A268 replaced with G, I, L, S, T, M, or V; Q269 replaced with N; I270 replaced with A, G, L, S, T, M, or V; S271 replaced with A, G, I, L, T, M, or V; L272 replaced with A, G, I, S, T, M, or V; D273 replaced with E; G274 replaced with A, I, L, S, T, M, or V; D275 replaced with E; V276 replaced with A, G, I, L, S, T, or M; T277 replaced with A, G, I, L, S, M, or V; F278 replaced with W, or Y; F279 replaced with W, or Y; G280 replaced with A, I, L, S, T, M, or V; A281 replaced with G, I, L, S, T, M, or V; L282 replaced with A, G, I, S, T, M, or V; K283 replaced with H, or R; L284 replaced with A, G, I, S, T, M, or V; and/or L285 replaced with A, G, I, S, T, M, or V. Polynucleotides encoding these polypeptides are also encompassed by the invention. The resulting Neutrokin-alpha proteins of the invention may be routinely screened for Neutrokin-alpha and/or Neutrokin-alphaSV functional activity and/or physical properties (such as, for example, enhanced or reduced stability and/or solubility). Preferably, the resulting proteins of the invention have an increased and/or a decreased Neutrokin-alpha and/or Neutrokin-alphaSV functional activity. More preferably, the resulting Neutrokin-alpha and/or Neutrokin-alphaSV proteins of the invention have more than one increased and/or decreased Neutrokin-alpha and/or Neutrokin-alpha SV functional activity and/or physical property.

[0336] In another embodiment, site directed changes at the amino acid level of Neutrokin-alphaSV can be made by replacing a particular amino acid with a conservative substitution. Preferred conservative substitution mutations of the Neutrokin-alphaSV amino acid sequence provided in SEQ ID NO:19 include: M1 replaced with A, G, I, L, S, T, or V; D2 replaced with E; D3 replaced with E; S4 replaced with A, G, I, L, T, M, or V; T5 replaced with A, G, I, L, S, M, or V; E6 replaced with D; R7 replaced with H, or K; E8 replaced with D; Q9 replaced with N; S10 replaced with A, G, I, L, T, M, or V; R11 replaced with H, or K; L12 replaced with A, G, I, S, T, M, or V; T13 replaced with A, G, I, L, S, M, or V; S14 replaced with A, G, I, L, T, M, or V; L16 replaced with A, G, I, S, T, M, or V; K17 replaced with H, or R; K18 replaced with H, or R; R19 replaced with H, or K; E20 replaced with D; E21 replaced with D; M22 replaced with A, G, I, L, S, T, or V; K23 replaced with H, or R; L24 replaced with A, G, I, S, T, M, or V; K25 replaced with H, or R; E26 replaced with D; V28 replaced with A, G, I, L, S, T, or M; S29 replaced with A, G, I, L, T, M, or V; I30 replaced with A, G, L, S, T, M, or V; L31 replaced with A, G,

I, S, T, M, or V; R33 replaced with H, or K; K34 replaced with H, or R; E35 replaced with D; S36 replaced with A, G, I, L, T, M, or V; S38 replaced with A, G, I, L, T, M, or V; V39 replaced with A, G, I, L, S, T, or M; R40 replaced with H, or K; S41 replaced with A, G, I, L, T, M, or V; S42 replaced with A, G, I, L, T, M, or V; K43 replaced with H, or R; D44 replaced with E; G45 replaced with A, I, L, S, T, M, or V; K46 replaced with H, or R; L47 replaced with A, G, I, S, T, M, or V; L48 replaced with A, G, I, S, T, M, or V; A49 replaced with G, I, L, S, T, M, or V; A50 replaced with G, I, L, S, T, M, or V; T51 replaced with A, G, I, L, S, M, or V; L52 replaced with A, G, I, S, T, M, or V; L53 replaced with A, G, I, S, T, M, or V; L54 replaced with A, G, I, S, T, M, or V; A55 replaced with G, I, L, S, T, M, or V; L56 replaced with A, G, I, S, T, M, or V; L57 replaced with A, G, I, S, T, M, or V; S58 replaced with A, G, I, L, T, M, or V; L61 replaced with A, G, I, S, T, M, or V; T62 replaced with A, G, I, L, S, M, or V; V63 replaced with A, G, I, L, S, T, or M; V64 replaced with A, G, I, L, S, T, or M; S65 replaced with A, G, I, L, T, M, or V; F66 replaced with W, or Y; Y67 replaced with F, or W; Q68 replaced with N; V69 replaced with A, G, I, L, S, T, or M; A70 replaced with G, I, L, S, T, M, or V; A71 replaced with G, I, L, S, T, M, or V; L72 replaced with A, G, I, S, T, M, or V; Q73 replaced with N; G74 replaced with A, I, L, S, T, M, or V; D75 replaced with E; L76 replaced with A, G, I, S, T, M, or V; A77 replaced with G, I, L, S, T, M, or V; S78 replaced with A, G, I, L, T, M, or V; L79 replaced with A, G, I, S, T, M, or V; R80 replaced with H, or K; A81 replaced with G, I, L, S, T, M, or V; E82 replaced with D; L83 replaced with A, G, I, S, T, M, or V; Q84 replaced with N; G85 replaced with A, I, L, S, T, M, or V; H86 replaced with K, or R; H87 replaced with K, or R; A88 replaced with G, I, L, S, T, M, or V; E89 replaced with D; K90 replaced with H, or R; L91 replaced with A, G, I, S, T, M, or V; A93 replaced with G, I, L, S, T, M, or V; G94 replaced with A, I, L, S, T, M, or V; A95 replaced with G, I, L, S, T, M, or V; G96 replaced with A, I, L, S, T, M, or V; A97 replaced with G, I, L, S, T, M, or V; K99 replaced with H, or R; A100 replaced with G, I, L, S, T, M, or V; G101 replaced with A, I, L, S, T, M, or V; L102 replaced with A, G, I, S, T, M, or V; E103 replaced with D; E104 replaced with D; A105 replaced with G, I, L, S, T, M, or V; A107 replaced with G, I, L, S, T, M, or V; V108 replaced with A, G, I, L, S, T, or M; T109 replaced with A, G, I, L, S, M, or V; A110 replaced with G, I, L, S, T, M, or V; G111 replaced with A, I, L, S, T, M, or V; L112 replaced with A, G, I, S, T, M, or V; K113 replaced with H, or R; I114 replaced with A, G, L, S, T, M, or V; F115

replaced with W, or Y; E116 replaced with D; A119 replaced with G, I, L, S, T, M, or V; G121 replaced with A, I, L, S, T, M, or V; E122 replaced with D; G123 replaced with A, I, L, S, T, M, or V; N124 replaced with Q; S125 replaced with A, G, I, L, T, M, or V; S126 replaced with A, G, I, L, T, M, or V; Q127 replaced with N; N128 replaced with Q; S129 replaced with A, G, I, L, T, M, or V; R130 replaced with H, or K; N131 replaced with Q; K132 replaced with H, or R; R133 replaced with H, or K; A134 replaced with G, I, L, S, T, M, or V; V135 replaced with A, G, I, L, S, T, or M; Q136 replaced with N; G137 replaced with A, I, L, S, T, M, or V; E139 replaced with D; E140 replaced with D; T141 replaced with A, G, I, L, S, M, or V; G142 replaced with A, I, L, S, T, M, or V; S143 replaced with A, G, I, L, T, M, or V; Y144 replaced with F, or W; T145 replaced with A, G, I, L, S, M, or V; F146 replaced with W, or Y; V147 replaced with A, G, I, L, S, T, or M; W149 replaced with F, or Y; L150 replaced with A, G, I, S, T, M, or V; L151 replaced with A, G, I, S, T, M, or V; S152 replaced with A, G, I, L, T, M, or V; F153 replaced with W, or Y; K154 replaced with H, or R; R155 replaced with H, or K; G156 replaced with A, I, L, S, T, M, or V; S157 replaced with A, G, I, L, T, M, or V; A158 replaced with G, I, L, S, T, M, or V; L159 replaced with A, G, I, S, T, M, or V; E160 replaced with D; E161 replaced with D; K162 replaced with H, or R; E163 replaced with D; N164 replaced with Q; K165 replaced with H, or R; I166 replaced with A, G, L, S, T, M, or V; L167 replaced with A, G, I, S, T, M, or V; V168 replaced with A, G, I, L, S, T, or M; K169 replaced with H, or R; E170 replaced with D; T171 replaced with A, G, I, L, S, M, or V; G172 replaced with A, I, L, S, T, M, or V; Y173 replaced with F, or W; F174 replaced with W, or Y; F175 replaced with W, or Y; I176 replaced with A, G, L, S, T, M, or V; Y177 replaced with F, or W; G178 replaced with A, I, L, S, T, M, or V; Q179 replaced with N; V180 replaced with A, G, I, L, S, T, or M; L181 replaced with A, G, I, S, T, M, or V; Y182 replaced with F, or W; T183 replaced with A, G, I, L, S, M, or V; D184 replaced with E; K185 replaced with H, or R; T186 replaced with A, G, I, L, S, M, or V; Y187 replaced with F, or W; A188 replaced with G, I, L, S, T, M, or V; M189 replaced with A, G, I, L, S, T, or V; G190 replaced with A, I, L, S, T, M, or V; H191 replaced with K, or R; L192 replaced with A, G, I, S, T, M, or V; I193 replaced with A, G, L, S, T, M, or V; Q194 replaced with N; R195 replaced with H, or K; K196 replaced with H, or R; K197 replaced with H, or R; V198 replaced with A, G, I, L, S, T, or M; H199 replaced with K, or R; V200 replaced with A, G, I, L, S, T, or M; F201 replaced with W, or Y;

G202 replaced with A, I, L, S, T, M, or V; D203 replaced with E; E204 replaced with D; L205 replaced with A, G, I, S, T, M, or V; S206 replaced with A, G, I, L, T, M, or V; L207 replaced with A, G, I, S, T, M, or V; V208 replaced with A, G, I, L, S, T, or M; T209 replaced with A, G, I, L, S, M, or V; L210 replaced with A, G, I, S, T, M, or V; F211 replaced with W, or Y; R212 replaced with H, or K; I214 replaced with A, G, L, S, T, M, or V; Q215 replaced with N; N216 replaced with Q; M217 replaced with A, G, I, L, S, T, or V; E219 replaced with D; T220 replaced with A, G, I, L, S, M, or V; L221 replaced with A, G, I, S, T, M, or V; N223 replaced with Q; N224 replaced with Q; S225 replaced with A, G, I, L, T, M, or V; Y227 replaced with F, or W; S228 replaced with A, G, I, L, T, M, or V; A229 replaced with G, I, L, S, T, M, or V; G230 replaced with A, I, L, S, T, M, or V; I231 replaced with A, G, L, S, T, M, or V; A232 replaced with G, I, L, S, T, M, or V; K233 replaced with H, or R; L234 replaced with A, G, I, S, T, M, or V; E235 replaced with D; E236 replaced with D; G237 replaced with A, I, L, S, T, M, or V; D238 replaced with E; E239 replaced with D; L240 replaced with A, G, I, S, T, M, or V; Q241 replaced with N; L242 replaced with A, G, I, S, T, M, or V; A243 replaced with G, I, L, S, T, M, or V; I244 replaced with A, G, L, S, T, M, or V; R246 replaced with H, or K; E247 replaced with D; N248 replaced with Q; A249 replaced with G, I, L, S, T, M, or V; Q250 replaced with N; I251 replaced with A, G, L, S, T, M, or V; S252 replaced with A, G, I, L, T, M, or V; L253 replaced with A, G, I, S, T, M, or V; D254 replaced with E; G255 replaced with A, I, L, S, T, M, or V; D256 replaced with E; V257 replaced with A, G, I, L, S, T, or M; T258 replaced with A, G, I, L, S, M, or V; F259 replaced with W, or Y; F260 replaced with W, or Y; G261 replaced with A, I, L, S, T, M, or V; A262 replaced with G, I, L, S, T, M, or V; L263 replaced with A, G, I, S, T, M, or V; K264 replaced with H, or R; L265 replaced with A, G, I, S, T, M, or V; and/or L266 replaced with A, G, I, S, T, M, or V. Polynucleotides encoding these polypeptides are also encompassed by the invention. The resulting Neutrokin- $\alpha$  proteins of the invention may be routinely screened for Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV functional activity and/or physical properties (such as, for example, enhanced or reduced stability and/or solubility). Preferably, the resulting proteins of the invention have an increased and/or a decreased Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV functional activity. More preferably, the resulting Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV proteins of the invention have

more than one increased and/or decreased Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$  SV functional activity and/or physical property.

[0337] In another embodiment, site directed changes at the amino acid level of Neutrokin- $\alpha$  can be made by replacing a particular amino acid with a conservative substitution. Preferred conservative substitution mutations of the Neutrokin- $\alpha$  amino acid sequence provided in SEQ ID NO:23 include: R1 replaced with H, or K; V2 replaced with A, G, I, L, S, T, or M; V3 replaced with A, G, I, L, S, T, or M; D4 replaced with E; L5 replaced with A, G, I, S, T, M, or V; S6 replaced with A, G, I, L, T, M, or V; A7 replaced with G, I, L, S, T, M, or V; A10 replaced with G, I, L, S, T, M, or V; L13 replaced with A, G, I, S, T, M, or V; G15 replaced with A, I, L, S, T, M, or V; R17 replaced with H, or K; H18 replaced with K, or R; S19 replaced with A, G, I, L, T, M, or V; Q20 replaced with N; H21 replaced with K, or R; D22 replaced with E; D23 replaced with E; N24 replaced with Q; G25 replaced with A, I, L, S, T, M, or V; M26 replaced with A, G, I, L, S, T, or V; N27 replaced with Q; L28 replaced with A, G, I, S, T, M, or V; R29 replaced with H, or K; N30 replaced with Q; R31 replaced with H, or K; T32 replaced with A, G, I, L, S, M, or V; Y33 replaced with F, or W; T34 replaced with A, G, I, L, S, M, or V; F35 replaced with W, or Y; V36 replaced with A, G, I, L, S, T, or M; W38 replaced with F, or Y; L39 replaced with A, G, I, S, T, M, or V; L40 replaced with A, G, I, S, T, M, or V; S41 replaced with A, G, I, L, T, M, or V; F42 replaced with W, or Y; K43 replaced with H, or R; R44 replaced with H, or K; G45 replaced with A, I, L, S, T, M, or V; N46 replaced with Q; A47 replaced with G, I, L, S, T, M, or V; L48 replaced with A, G, I, S, T, M, or V; E49 replaced with D; E50 replaced with D; K51 replaced with H, or R; E52 replaced with D; N53 replaced with Q; K54 replaced with H, or R; I55 replaced with A, G, L, S, T, M, or V; V56 replaced with A, G, I, L, S, T, or M; V57 replaced with A, G, I, L, S, T, or M; R58 replaced with H, or K; Q59 replaced with N; T60 replaced with A, G, I, L, S, M, or V; G61 replaced with A, I, L, S, T, M, or V; Y62 replaced with F, or W; F63 replaced with W, or Y; F64 replaced with W, or Y; I65 replaced with A, G, L, S, T, M, or V; Y66 replaced with F, or W; S67 replaced with A, G, I, L, T, M, or V; Q68 replaced with N; V69 replaced with A, G, I, L, S, T, or M; L70 replaced with A, G, I, S, T, M, or V; Y71 replaced with F, or W; T72 replaced with A, G, I, L, S, M, or V; D73 replaced with E; I75 replaced with A, G, L, S, T, M, or V; F76 replaced with W, or Y; A77 replaced with G, I, L, S, T, M, or V; M78 replaced with A, G, I, L, S, T, or V; G79

replaced with A, I, L, S, T, M, or V; H80 replaced with K, or R; V81 replaced with A, G, I, L, S, T, or M; I82 replaced with A, G, L, S, T, M, or V; Q83 replaced with N; R84 replaced with H, or K; K85 replaced with H, or R; K86 replaced with H, or R; V87 replaced with A, G, I, L, S, T, or M; H88 replaced with K, or R; V89 replaced with A, G, I, L, S, T, or M; F90 replaced with W, or Y; G91 replaced with A, I, L, S, T, M, or V; D92 replaced with E; E93 replaced with D; L94 replaced with A, G, I, S, T, M, or V; S95 replaced with A, G, I, L, T, M, or V; L96 replaced with A, G, I, S, T, M, or V; V97 replaced with A, G, I, L, S, T, or M; T98 replaced with A, G, I, L, S, M, or V; L99 replaced with A, G, I, S, T, M, or V; F100 replaced with W, or Y; R101 replaced with H, or K; I103 replaced with A, G, L, S, T, M, or V; Q104 replaced with N; N105 replaced with Q; M106 replaced with A, G, I, L, S, T, or V; K108 replaced with H, or R; T109 replaced with A, G, I, L, S, M, or V; L110 replaced with A, G, I, S, T, M, or V; N112 replaced with Q; N113 replaced with Q; S114 replaced with A, G, I, L, T, M, or V; Y116 replaced with F, or W; S117 replaced with A, G, I, L, T, M, or V; A118 replaced with G, I, L, S, T, M, or V; G119 replaced with A, I, L, S, T, M, or V; I120 replaced with A, G, L, S, T, M, or V; A121 replaced with G, I, L, S, T, M, or V; R122 replaced with H, or K; L123 replaced with A, G, I, S, T, M, or V; E124 replaced with D; E125 replaced with D; G126 replaced with A, I, L, S, T, M, or V; D127 replaced with E; E128 replaced with D; I129 replaced with A, G, L, S, T, M, or V; Q130 replaced with N; L131 replaced with A, G, I, S, T, M, or V; A132 replaced with G, I, L, S, T, M, or V; I133 replaced with A, G, L, S, T, M, or V; R135 replaced with H, or K; E136 replaced with D; N137 replaced with Q; A138 replaced with G, I, L, S, T, M, or V; Q139 replaced with N; I140 replaced with A, G, L, S, T, M, or V; S141 replaced with A, G, I, L, T, M, or V; R142 replaced with H, or K; N143 replaced with Q; G144 replaced with A, I, L, S, T, M, or V; D145 replaced with E; D146 replaced with E; T147 replaced with A, G, I, L, S, M, or V; F148 replaced with W, or Y; F149 replaced with W, or Y; G150 replaced with A, I, L, S, T, M, or V; A151 replaced with G, I, L, S, T, M, or V; L152 replaced with A, G, I, S, T, M, or V; K153 replaced with H, or R; L154 replaced with A, G, I, S, T, M, or V; and/or L155 replaced with A, G, I, S, T, M, or V. Polynucleotides encoding these polypeptides are also encompassed by the invention. The resulting Neutrokin- $\alpha$  proteins of the invention may be routinely screened for Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV functional activity and/or physical properties (such as, for example, enhanced or reduced stability

and/or solubility). Preferably, the resulting proteins of the invention have an increased and/or a decreased Neutrokin-alpha and/or Neutrokin-alphaSV functional activity. More preferably, the resulting Neutrokin-alpha and/or Neutrokin-alphaSV proteins of the invention have more than one increased and/or decreased Neutrokin-alpha and/or Neutrokin-alpha SV functional activity and/or physical property.

**[0338]** In another embodiment, site directed changes at the amino acid level of Neutrokin-alpha can be made by replacing a particular amino acid with a conservative substitution. Preferred conservative substitution mutations of the Neutrokin-alpha amino acid sequence provided in SEQ ID NO:38 include: M1 replaced with A, G, I, L, S, T, or V; D2 replaced with E; E3 replaced with D; S4 replaced with A, G, I, L, T, M, or V; A5 replaced with G, I, L, S, T, M, or V; K6 replaced with H, or R; T7 replaced with A, G, I, L, S, M, or V; L8 replaced with A, G, I, S, T, M, or V; L13 replaced with A, G, I, S, T, M, or V; F15 replaced with W, or Y; S17 replaced with A, G, I, L, T, M, or V; E18 replaced with D; K19 replaced with H, or R; G20 replaced with A, I, L, S, T, M, or V; E21 replaced with D; D22 replaced with E; M23 replaced with A, G, I, L, S, T, or V; K24 replaced with H, or R; V25 replaced with A, G, I, L, S, T, or M; G26 replaced with A, I, L, S, T, M, or V; Y27 replaced with F, or W; D28 replaced with E; I30 replaced with A, G, L, S, T, M, or V; T31 replaced with A, G, I, L, S, M, or V; Q33 replaced with N; K34 replaced with H, or R; E35 replaced with D; E36 replaced with D; G37 replaced with A, I, L, S, T, M, or V; A38 replaced with G, I, L, S, T, M, or V; W39 replaced with F, or Y; F40 replaced with W, or Y; G41 replaced with A, I, L, S, T, M, or V; I42 replaced with A, G, L, S, T, M, or V; R44 replaced with H, or K; D45 replaced with E; G46 replaced with A, I, L, S, T, M, or V; R47 replaced with H, or K; L48 replaced with A, G, I, S, T, M, or V; L49 replaced with A, G, I, S, T, M, or V; A50 replaced with G, I, L, S, T, M, or V; A51 replaced with G, I, L, S, T, M, or V; T52 replaced with A, G, I, L, S, M, or V; L53 replaced with A, G, I, S, T, M, or V; L54 replaced with A, G, I, S, T, M, or V; L55 replaced with A, G, I, S, T, M, or V; A56 replaced with G, I, L, S, T, M, or V; L57 replaced with A, G, I, S, T, M, or V; L58 replaced with A, G, I, S, T, M, or V; S59 replaced with A, G, I, L, T, M, or V; S60 replaced with A, G, I, L, T, M, or V; S61 replaced with A, G, I, L, T, M, or V; F62 replaced with W, or Y; T63 replaced with A, G, I, L, S, M, or V; A64 replaced with G, I, L, S, T, M, or V; M65 replaced with A, G, I, L, S, T, or V; S66 replaced with A, G, I, L, T, M, or V; L67 replaced with A, G, I, S, T, M,



or V; Y68 replaced with F, or W; Q69 replaced with N; L70 replaced with A, G, I, S, T, M, or V; A71 replaced with G, I, L, S, T, M, or V; A72 replaced with G, I, L, S, T, M, or V; L73 replaced with A, G, I, S, T, M, or V; Q74 replaced with N; A75 replaced with G, I, L, S, T, M, or V; D76 replaced with E; L77 replaced with A, G, I, S, T, M, or V; M78 replaced with A, G, I, L, S, T, or V; N79 replaced with Q; L80 replaced with A, G, I, S, T, M, or V; R81 replaced with H, or K; M82 replaced with A, G, I, L, S, T, or V; E83 replaced with D; L84 replaced with A, G, I, S, T, M, or V; Q85 replaced with N; S86 replaced with A, G, I, L, T, M, or V; Y87 replaced with F, or W; R88 replaced with H, or K; G89 replaced with A, I, L, S, T, M, or V; S90 replaced with A, G, I, L, T, M, or V; A91 replaced with G, I, L, S, T, M, or V; T92 replaced with A, G, I, L, S, M, or V; A94 replaced with G, I, L, S, T, M, or V; A95 replaced with G, I, L, S, T, M, or V; A96 replaced with G, I, L, S, T, M, or V; G97 replaced with A, I, L, S, T, M, or V; A98 replaced with G, I, L, S, T, M, or V; E100 replaced with D; L101 replaced with A, G, I, S, T, M, or V; T102 replaced with A, G, I, L, S, M, or V; A103 replaced with G, I, L, S, T, M, or V; G104 replaced with A, I, L, S, T, M, or V; V105 replaced with A, G, I, L, S, T, or M; K106 replaced with H, or R; L107 replaced with A, G, I, S, T, M, or V; L108 replaced with A, G, I, S, T, M, or V; T109 replaced with A, G, I, L, S, M, or V; A111 replaced with G, I, L, S, T, M, or V; A112 replaced with G, I, L, S, T, M, or V; R114 replaced with H, or K; H116 replaced with K, or R; N117 replaced with Q; S118 replaced with A, G, I, L, T, M, or V; S119 replaced with A, G, I, L, T, M, or V; R120 replaced with H, or K; G121 replaced with A, I, L, S, T, M, or V; H122 replaced with K, or R; R123 replaced with H, or K; N124 replaced with Q; R125 replaced with H, or K; R126 replaced with H, or K; A127 replaced with G, I, L, S, T, M, or V; F128 replaced with W, or Y; Q129 replaced with N; G130 replaced with A, I, L, S, T, M, or V; E132 replaced with D; E133 replaced with D; T134 replaced with A, G, I, L, S, M, or V; E135 replaced with D; Q136 replaced with N; D137 replaced with E; V138 replaced with A, G, I, L, S, T, or M; D139 replaced with E; L140 replaced with A, G, I, S, T, M, or V; S141 replaced with A, G, I, L, T, M, or V; A142 replaced with G, I, L, S, T, M, or V; A145 replaced with G, I, L, S, T, M, or V; L148 replaced with A, G, I, S, T, M, or V; G150 replaced with A, I, L, S, T, M, or V; R152 replaced with H, or K; H153 replaced with K, or R; S154 replaced with A, G, I, L, T, M, or V; Q155 replaced with N; H156 replaced with K, or R; D157 replaced with E; D158 replaced with E; N159 replaced with Q; G160 replaced with A, I, L, S, T,

M, or V; M161 replaced with A, G, I, L, S, T, or V; N162 replaced with Q; L163 replaced with A, G, I, S, T, M, or V; R164 replaced with H, or K; N165 replaced with Q; I166 replaced with A, G, L, S, T, M, or V; I167 replaced with A, G, L, S, T, M, or V; Q168 replaced with N; D169 replaced with E; L171 replaced with A, G, I, S, T, M, or V; Q172 replaced with N; L173 replaced with A, G, I, S, T, M, or V; I174 replaced with A, G, L, S, T, M, or V; A175 replaced with G, I, L, S, T, M, or V; D176 replaced with E; S177 replaced with A, G, I, L, T, M, or V; D178 replaced with E; T179 replaced with A, G, I, L, S, M, or V; A181 replaced with G, I, L, S, T, M, or V; L182 replaced with A, G, I, S, T, M, or V; E183 replaced with D; E184 replaced with D; K185 replaced with H, or R; E186 replaced with D; N187 replaced with Q; K188 replaced with H, or R; I189 replaced with A, G, L, S, T, M, or V; V190 replaced with A, G, I, L, S, T, or M; V191 replaced with A, G, I, L, S, T, or M; R192 replaced with H, or K; Q193 replaced with N; T194 replaced with A, G, I, L, S, M, or V; G195 replaced with A, I, L, S, T, M, or V; Y196 replaced with F, or W; F197 replaced with W, or Y; F198 replaced with W, or Y; I199 replaced with A, G, L, S, T, M, or V; Y200 replaced with F, or W; S201 replaced with A, G, I, L, T, M, or V; Q202 replaced with N; V203 replaced with A, G, I, L, S, T, or M; L204 replaced with A, G, I, S, T, M, or V; Y205 replaced with F, or W; T206 replaced with A, G, I, L, S, M, or V; D207 replaced with E; I209 replaced with A, G, L, S, T, M, or V; F210 replaced with W, or Y; A211 replaced with G, I, L, S, T, M, or V; M212 replaced with A, G, I, L, S, T, or V; G213 replaced with A, I, L, S, T, M, or V; H214 replaced with K, or R; V215 replaced with A, G, I, L, S, T, or M; I216 replaced with A, G, L, S, T, M, or V; Q217 replaced with N; R218 replaced with H, or K; K219 replaced with H, or R; K220 replaced with H, or R; V221 replaced with A, G, I, L, S, T, or M; H222 replaced with K, or R; V223 replaced with A, G, I, L, S, T, or M; F224 replaced with W, or Y; G225 replaced with A, I, L, S, T, M, or V; D226 replaced with E; E227 replaced with D; L228 replaced with A, G, I, S, T, M, or V; S229 replaced with A, G, I, L, T, M, or V; L230 replaced with A, G, I, S, T, M, or V; V231 replaced with A, G, I, L, S, T, or M; T232 replaced with A, G, I, L, S, M, or V; L233 replaced with A, G, I, S, T, M, or V; F234 replaced with W, or Y; R235 replaced with H, or K; I237 replaced with A, G, L, S, T, M, or V; Q238 replaced with N; N239 replaced with Q; M240 replaced with A, G, I, L, S, T, or V; K242 replaced with H, or R; T243 replaced with A, G, I, L, S, M, or V; L244 replaced with A, G, I, S, T, M, or V; N246 replaced with Q; N247 replaced with Q; S248 replaced with A, G, I, L, T,

M, or V; Y250 replaced with F, or W; S251 replaced with A, G, I, L, T, M, or V; A252 replaced with G, I, L, S, T, M, or V; G253 replaced with A, I, L, S, T, M, or V; I254 replaced with A, G, L, S, T, M, or V; A255 replaced with G, I, L, S, T, M, or V; R256 replaced with H, or K; L257 replaced with A, G, I, S, T, M, or V; E258 replaced with D; E259 replaced with D; G260 replaced with A, I, L, S, T, M, or V; D261 replaced with E; E262 replaced with D; I263 replaced with A, G, L, S, T, M, or V; Q264 replaced with N; L265 replaced with A, G, I, S, T, M, or V; A266 replaced with G, I, L, S, T, M, or V; I267 replaced with A, G, L, S, T, M, or V; R269 replaced with H, or K; E270 replaced with D; N271 replaced with Q; A272 replaced with G, I, L, S, T, M, or V; Q273 replaced with N; I274 replaced with A, G, L, S, T, M, or V; S275 replaced with A, G, I, L, T, M, or V; R276 replaced with H, or K; N277 replaced with Q; G278 replaced with A, I, L, S, T, M, or V; D279 replaced with E; D280 replaced with E; T281 replaced with A, G, I, L, S, M, or V; F282 replaced with W, or Y; F283 replaced with W, or Y; G284 replaced with A, I, L, S, T, M, or V; A285 replaced with G, I, L, S, T, M, or V; L286 replaced with A, G, I, S, T, M, or V; K287 replaced with H, or R; L288 replaced with A, G, I, S, T, M, or V; and/or L289 replaced with A, G, I, S, T, M, or V. Polynucleotides encoding these polypeptides are also encompassed by the invention. The resulting Neutrokin-alpha proteins of the invention may be routinely screened for Neutrokin-alpha and/or Neutrokin-alphaSV functional activity and/or physical properties (such as, for example, enhanced or reduced stability and/or solubility). Preferably, the resulting proteins of the invention have an increased and/or a decreased Neutrokin-alpha and/or Neutrokin-alphaSV functional activity. More preferably, the resulting Neutrokin-alpha and/or Neutrokin-alphaSV proteins of the invention have more than one increased and/or decreased Neutrokin-alpha and/or Neutrokin-alpha SV functional activity and/or physical property.

[0339] Amino acids in the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science* 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for functional activity, such ligand binding and the ability to stimulate lymphocyte (e.g., B cell) as, for example, proliferation, differentiation, and/or activation.

**[0340]** Of special interest are substitutions of charged amino acids with other charged or neutral amino acids which may produce proteins with highly desirable improved characteristics, such as less aggregation. Aggregation may not only reduce activity but also be problematic when preparing pharmaceutical formulations, because aggregates can be immunogenic (Pinckard *et al.*, *Clin. Exp. Immunol.* 2:331-340 (1967); Robbins *et al.*, *Diabetes* 36: 838-845 (1987); Cleland *et al.*, *Crit. Rev. Therapeutic Drug Carrier Systems* 10:307-377 (1993).

**[0341]** In another embodiment, the invention provides for polypeptides having amino acid sequences containing non-conservative substitutions of the amino acid sequence provided in SEQ ID NO:2. For example, non-conservative substitutions of the Neutrokin- $\alpha$  protein sequence provided in SEQ ID NO:2 include: M1 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D2 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D3 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S4 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T5 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E6 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R7 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E8 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q9 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S10 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R11 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L12 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T13 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S14 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C15 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; L16 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K17 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K18 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R19 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E20 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E21 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; M22 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K23 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L24 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K25 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E26 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C27 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; V28 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S29 replaced with D, E, H,

K, R, N, Q, F, W, Y, P, or C; I30 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L31 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P32 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; R33 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K34 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E35 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S36 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P37 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; S38 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V39 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R40 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S41 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S42 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K43 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D44 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G45 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K46 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L47 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L48 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A49 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A50 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T51 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L52 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L53 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L54 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A55 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L56 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L57 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S58 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C59 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; C60 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; L61 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T62 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V63 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V64 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S65 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F66 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Y67 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q68 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; V69 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A70 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A71 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L72 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q73 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G74 replaced with D, E, H, K, R, N, Q, F, W, Y, P,

or C; D75 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L76 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A77 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S78 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L79 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R80 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A81 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E82 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L83 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q84 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G85 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H86 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; H87 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A88 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E89 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K90 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L91 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P92 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; A93 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G94 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A95 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G96 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A97 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P98 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; K99 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A100 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G101 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L102 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E103 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E104 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A105 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P106 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; A107 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V108 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T109 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A110 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G111 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L112 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K113 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I114 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F115 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; E116 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P117 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; P118 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V,

N, Q, F, W, Y, or C; A119 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P120 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G121 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E122 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G123 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N124 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S125 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S126 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q127 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; N128 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S129 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R130 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N131 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; K132 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R133 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A134 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V135 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q136 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G137 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P138 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; E139 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E140 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T141 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V142 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T143 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q144 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; D145 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C146 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; L147 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q148 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L149 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I150 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A151 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D152 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S153 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E154 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T155 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P156 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; T157 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I158 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q159 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; K160 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G161

replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S162 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y163 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T164 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F165 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; V166 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P167 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; W168 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L169 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L170 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S171 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F172 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; K173 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R174 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G175 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S176 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A177 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L178 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E179 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E180 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K181 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E182 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N183 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; K184 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I185 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L186 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V187 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K188 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E189 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T190 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G191 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y192 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F193 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F194 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; I195 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y196 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G197 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q198 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; V199 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L200 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y201 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T202 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D203 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K204 replaced with D, E, A, G, I, L,



S, T, M, V, N, Q, F, W, Y, P, or C; T205 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y206 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A207 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M208 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G209 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H210 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L211 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I212 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q213 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; R214 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K215 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K216 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V217 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H218 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V219 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F220 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G221 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D222 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E223 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L224 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S225 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L226 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V227 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T228 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L229 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F230 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; R231 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C232 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; I233 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q234 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; N235 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; M236 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P237 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; E238 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T239 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L240 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P241 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; N242 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; N243 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S244 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C245 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; Y246 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C;

S247 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A248 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G249 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I250 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A251 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K252 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L253 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E254 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E255 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G256 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D257 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E258 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L259 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q260 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L261 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A262 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I263 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P264 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; R265 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E266 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N267 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; A268 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q269 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I270 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S271 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L272 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D273 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G274 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D275 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V276 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T277 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F278 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F279 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G280 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A281 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L282 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K283 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L284 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; and/or L285 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C. Polynucleotides encoding these polypeptides are also encompassed by the invention. The resulting Neutrokin- $\alpha$  proteins of the invention may be routinely screened for Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV functional activities and/or physical properties (such as, for example, enhanced or reduced

stability and/or solubility) described throughout the specification and known in the art. Preferably, the resulting proteins of the invention have an increased and/or a decreased Neutrokin-alpha and/or Neutrokin-alphaSV functional activity. More preferably, the resulting Neutrokin-alpha and/or Neutrokin-alphaSV proteins of the invention have more than one increased and/or decreased Neutrokin-alpha and/or Neutrokin-alphaSV functional activity and/or physical property.

**[0342]** In an additional embodiment, Neutrokin-alpha polypeptides of the invention comprise, or alternatively consist of, more than one amino acid (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 and 50) replaced with the substituted amino acids as described above (either conservative or nonconservative).

**[0343]** In another embodiment of the invention, non-conservative substitutions of the Neutrokin-alphaSV protein sequence provided in SEQ ID NO:19 include: M1 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D2 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D3 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S4 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T5 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E6 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R7 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E8 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q9 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S10 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R11 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L12 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T13 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S14 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C15 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; L16 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K17 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K18 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R19 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E20 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E21 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; M22 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K23 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L24 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K25 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E26 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C27 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y,

or P; V28 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S29 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I30 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L31 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P32 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; R33 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K34 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E35 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S36 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P37 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; S38 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V39 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R40 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S41 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S42 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K43 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D44 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G45 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K46 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L47 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L48 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A49 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A50 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T51 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L52 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L53 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L54 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A55 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L56 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L57 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S58 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C59 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; C60 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; L61 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T62 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V63 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V64 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S65 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F66 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Y67 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q68 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; V69 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A70 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A71 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L72 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q73 replaced with D, E, H, K, R, A,

G, I, L, S, T, M, V, F, W, Y, P, or C; G74 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D75 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L76 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A77 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S78 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L79 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R80 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A81 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E82 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L83 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q84 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G85 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H86 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; H87 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A88 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E89 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K90 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L91 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P92 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; A93 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G94 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A95 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G96 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A97 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P98 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; K99 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A100 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G101 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L102 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E103 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E104 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A105 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P106 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; A107 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V108 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T109 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A110 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G111 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L112 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K113 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I114 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F115 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; E116 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P117 replaced with D, E, H, K, R, A, G, I, L,

S, T, M, V, N, Q, F, W, Y, or C; P118 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; A119 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P120 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G121 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E122 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G123 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N124 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S125 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S126 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q127 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; N128 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S129 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R130 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N131 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; K132 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R133 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A134 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V135 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q136 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G137 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P138 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; E139 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E140 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T141 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G142 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S143 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y144 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T145 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F146 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; V147 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P148 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; W149 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L150 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L151 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S152 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F153 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; K154 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R155 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G156 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S157 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A158 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L159 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E160 replaced with H, K, R,

A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E161 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K162 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E163 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N164 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; K165 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I166 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L167 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V168 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K169 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E170 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T171 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G172 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y173 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F174 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F175 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; I176 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y177 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G178 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q179 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; V180 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L181 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y182 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T183 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D184 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K185 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T186 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y187 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A188 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M189 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G190 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H191 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L192 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I193 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q194 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; R195 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K196 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K197 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V198 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H199 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V200 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F201 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G202 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D203

replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E204 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L205 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S206 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L207 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V208 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T209 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L210 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F211 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; R212 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C213 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; I214 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q215 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; N216 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; M217 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P218 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; E219 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T220 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L221 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P222 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; N223 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; N224 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S225 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C226 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; Y227 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S228 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A229 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G230 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I231 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A232 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K233 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L234 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E235 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E236 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G237 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D238 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E239 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L240 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q241 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L242 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A243 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I244 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P245 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W,



Y, or C; R246 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E247 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N248 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; A249 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q250 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I251 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S252 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L253 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D254 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G255 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D256 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V257 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T258 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F259 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F260 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G261 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A262 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L263 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K264 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L265 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; and/or L266 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C. Polynucleotides encoding these polypeptides are also encompassed by the invention. The resulting Neutrokin-alpha proteins of the invention may be routinely screened for Neutrokin-alpha and/or Neutrokin-alphaSV functional activities and/or physical properties (such as, for example, enhanced or reduced stability and/or solubility) described throughout the specification and known in the art. Preferably, the resulting proteins of the invention have an increased and/or a decreased Neutrokin-alpha and/or Neutrokin-alphaSV functional activity. More preferably, the resulting Neutrokin-alpha and/or Neutrokin-alphaSV proteins of the invention have more than one increased and/or decreased Neutrokin-alpha and/or Neutrokin-alphaSV functional activity and/or physical property.

**[0344]** In an additional embodiment, Neutrokin-alpha polypeptides of the invention comprise, or alternatively consist of, more than one amino acid (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 and 50) replaced with the substituted amino acids as described above (either conservative or nonconservative).

**[0345]** For example, preferred non-conservative substitutions of the Neutrokin-alpha protein sequence provided in SEQ ID NO:23 include: R1 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V2 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C;

V3 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D4 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L5 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S6 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A7 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P8 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; P9 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; A10 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P11 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; C12 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; L13 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P14 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G15 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C16 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; R17 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; H18 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S19 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q20 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; H21 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D22 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D23 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N24 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G25 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M26 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N27 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L28 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R29 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N30 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; R31 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T32 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y33 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T34 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F35 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; V36 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P37 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; W38 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L39 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L40 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S41 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F42 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; K43 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R44 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G45 replaced with D, E, H, K, R, N, Q, F, W, Y, P,

or C; N46 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; A47 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L48 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E49 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E50 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K51 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E52 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N53 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; K54 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I55 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V56 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V57 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R58 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q59 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; T60 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G61 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y62 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F63 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F64 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; I65 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y66 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S67 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q68 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; V69 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L70 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y71 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T72 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D73 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P74 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; I75 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F76 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A77 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M78 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G79 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H80 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V81 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I82 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q83 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; R84 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K85 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K86 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V87 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H88 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V89 replaced with

D, E, H, K, R, N, Q, F, W, Y, P, or C; F90 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G91 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D92 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E93 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L94 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S95 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L96 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V97 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T98 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L99 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F100 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; R101 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C102 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; I103 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q104 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; N105 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; M106 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P107 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; K108 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T109 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L110 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P111 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; N112 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; N113 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S114 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C115 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; Y116 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S117 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A118 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G119 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I120 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A121 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R122 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L123 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E124 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E125 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G126 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D127 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E128 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I129 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q130 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L131 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A132

replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I133 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P134 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; R135 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E136 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N137 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; A138 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q139 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I140 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S141 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R142 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N143 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G144 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D145 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D146 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T147 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F148 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F149 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G150 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A151 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L152 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K153 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L154 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; and/or L155 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C. Polynucleotides encoding these polypeptides are also encompassed by the invention. The resulting Neutrokin-alpha proteins of the invention may be routinely screened for Neutrokin-alpha and/or Neutrokin-alphaSV functional activities and/or physical properties (such as, for example, enhanced or reduced stability and/or solubility) described throughout the specification and known in the art. Preferably, the resulting proteins of the invention have an increased and/or a decreased Neutrokin-alpha and/or Neutrokin-alphaSV functional activity. More preferably, the resulting Neutrokin-alpha and/or Neutrokin-alphaSV proteins of the invention have more than one increased and/or decreased Neutrokin-alpha and/or Neutrokin-alphaSV functional activity and/or physical property.

**[0346]** In an additional embodiment, Neutrokin-alpha polypeptides of the invention comprise, or alternatively consist of, more than one amino acid (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 and 50) replaced with the substituted amino acids as described above (either conservative or nonconservative).

[0347] For example, preferred non-conservative substitutions of the Neutrokin- $\alpha$  protein sequence provided in SEQ ID NO:38 include: M1 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D2 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E3 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S4 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A5 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K6 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T7 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L8 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P9 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; P10 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; P11 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; C12 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; L13 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C14 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; F15 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; C16 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; S17 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E18 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K19 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G20 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E21 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D22 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; M23 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K24 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V25 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G26 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y27 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; D28 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P29 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; I30 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T31 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P32 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; Q33 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; K34 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E35 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E36 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G37 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A38 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W39 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F40 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G41 replaced

with D, E, H, K, R, N, Q, F, W, Y, P, or C; I42 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C43 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R44 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D45 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G46 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R47 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L48 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L49 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A50 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A51 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T52 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L53 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L54 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L55 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A56 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L57 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L58 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S59 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S60 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S61 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F62 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T63 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A64 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M65 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S66 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L67 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y68 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q69 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L70 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A71 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A72 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L73 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q74 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; A75 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D76 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L77 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M78 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N79 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L80 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R81 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; M82 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E83 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L84 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q85 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S86 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y87

replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; R88 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G89 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S90 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A91 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T92 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P93 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; A94 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A95 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A96 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G97 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A98 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P99 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; E100 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L101 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T102 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A103 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G104 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V105 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K106 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L107 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L108 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T109 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P110 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; A111 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A112 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P113 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; R114 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P115 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; H116 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N117 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S118 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S119 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R120 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G121 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H122 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R123 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N124 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; R125 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R126 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A127 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F128 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q129 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G130 replaced with D, E, H, K, R, N,



Q, F, W, Y, P, or C; P131 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; E132 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E133 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T134 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E135 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q136 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; D137 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V138 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D139 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L140 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S141 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A142 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P143 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; P144 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; A145 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P146 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; C147 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; L148 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P149 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G150 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C151 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; R152 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; H153 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S154 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q155 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; H156 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D157 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D158 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N159 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G160 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M161 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N162 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L163 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R164 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N165 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I166 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I167 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q168 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; D169 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C170 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; L171 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C;

Q172 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L173 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I174 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A175 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D176 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S177 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D178 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T179 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P180 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; A181 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L182 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E183 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E184 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K185 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E186 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N187 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; K188 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I189 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V190 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V191 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R192 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q193 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; T194 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G195 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y196 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F197 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F198 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; I199 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y200 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S201 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q202 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; V203 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L204 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y205 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T206 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D207 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P208 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; I209 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F210 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A211 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M212 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G213 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H214 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or

C; V215 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I216 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q217 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; R218 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K219 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K220 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V221 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H222 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V223 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F224 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G225 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D226 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E227 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L228 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S229 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L230 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V231 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T232 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L233 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F234 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; R235 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C236 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; I237 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q238 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; N239 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; M240 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P241 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; K242 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T243 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L244 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P245 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; N246 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; N247 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S248 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C249 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; Y250 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S251 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A252 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G253 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I254 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A255 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R256 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L257 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or

C; E258 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E259 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G260 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D261 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E262 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I263 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q264 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L265 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A266 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I267 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P268 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; R269 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E270 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N271 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; A272 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q273 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I274 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S275 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R276 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N277 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G278 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D279 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D280 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T281 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F282 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F283 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G284 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A285 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L286 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K287 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L288 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; and/or L289 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C. Polynucleotides encoding these polypeptides are also encompassed by the invention. The resulting Neutrokin- $\alpha$  proteins of the invention may be routinely screened for Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV functional activities and/or physical properties (such as, for example, enhanced or reduced stability and/or solubility) described throughout the specification and known in the art. Preferably, the resulting proteins of the invention have an increased and/or a decreased Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV functional activity. More preferably, the resulting Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV proteins of the invention have more than one increased and/or

decreased Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV functional activity and/or physical property.

**[0348]** In an additional embodiment, Neutrokin- $\alpha$  polypeptides of the invention comprise, or alternatively consist of, more than one amino acid (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 and 50) replaced with the substituted amino acids as described above (either conservative or nonconservative).

**[0349]** Replacement of amino acids can also change the selectivity of the binding of a ligand to cell surface receptors. For example, Ostade *et al.*, *Nature* 361:266-268 (1993) describes certain mutations resulting in selective binding of TNF- $\alpha$  to only one of the two known types of TNF receptors. Since Neutrokin- $\alpha$  and Neutrokin- $\alpha$ SV are members of the TNF polypeptide family, mutations similar to those in TNF- $\alpha$  are likely to have similar effects in Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV.

**[0350]** Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith *et al.*, *J. Mol. Biol.* 224:899-904 (1992) and de Vos *et al.* *Science* 255:306-312 (1992)).

**[0351]** Since Neutrokin- $\alpha$  is a member of the TNF-related protein family, to modulate rather than completely eliminate functional activities (e.g., biological activities) of Neutrokin- $\alpha$ , mutations may be made in sequences encoding amino acids in the TNF conserved domain, i.e., in positions Gly-191 through Leu-284 of Figures 1A and 1B (SEQ ID NO:2), more preferably in residues within this region which are not conserved in all, most or several members of the TNF family (e.g., TNF- $\alpha$ , TNF- $\beta$ , LT- $\beta$ , and Fas Ligand) (see e.g., Figures 2A, 2B, 2C, and 2D). By making a specific mutation in Neutrokin- $\alpha$  in the position where such a conserved amino acid is typically found in related TNFs, the Neutrokin- $\alpha$  mutein will act as an antagonist, thus possessing activity for example, which inhibits lymphocyte (e.g., B cell) proliferation, differentiation, and/or activation. Accordingly, polypeptides of the present invention include Neutrokin- $\alpha$  mutants. Such Neutrokin- $\alpha$  mutants comprise, or alternatively consist of, fragments, variants or derivatives of the full-length or preferably the extracellular domain of the Neutrokin- $\alpha$  amino acid sequence shown in Figures 1A and 1B (SEQ ID NO:2). Polynucleotides encoding the above Neutrokin- $\alpha$  mutants are also encompassed by the invention.

[0352] Since Neutrokin- $\alpha$ SV is a member of the TNF-related protein family, to modulate rather than completely eliminate functional activities (e.g., biological activities) of Neutrokin- $\alpha$ SV, mutations may be made in sequences encoding amino acids in the TNF conserved domain, i.e., in positions Gly-172 through Leu-265 of Figures 5A and 5B (SEQ ID NO:19), more preferably in residues within this region which are not conserved in all, most or several members of the TNF family (e.g., TNF- $\alpha$ , TNF- $\beta$ , LT- $\beta$ , and Fas Ligand) (see e.g., Figures 2A 2B, 2C and 2D). By making a specific mutation in Neutrokin- $\alpha$ SV in the position where such a conserved amino acid is typically found in related TNFs, the Neutrokin- $\alpha$ SV mutein will act as an antagonist, thus possessing activity for example, which inhibits lymphocyte (e.g., B cell) proliferation, differentiation, and/or activation. Accordingly, polypeptides of the present invention include Neutrokin- $\alpha$ SV mutants. Such Neutrokin- $\alpha$ SV mutants comprise, or alternatively consist of, fragments, variants or derivatives of the full-length or preferably the extracellular domain of the Neutrokin- $\alpha$ SV amino acid sequence shown in Figures 5A and 5B (SEQ ID NO:19). Polynucleotides encoding the above Neutrokin- $\alpha$ SV mutants are also encompassed by the invention.

[0353] In addition, it will be recognized by one of ordinary skill in the art that mutations targeted to regions of a Neutrokin- $\alpha$  polypeptide of the invention which encompass the nineteen amino acid residue insertion which is not found in the Neutrokin- $\alpha$ SV polypeptide sequence (i.e., amino acid residues Val-142 through Lys-160 of the sequence presented in Figures 1A and 1B and in SEQ ID NO:2) may affect the observed functional activities (e.g., biological activity) of the Neutrokin- $\alpha$  polypeptide. More specifically, a partial, non-limiting and non-exclusive list of such residues of the Neutrokin- $\alpha$  polypeptide sequence which may be targeted for mutation includes the following amino acid residues of the Neutrokin- $\alpha$  polypeptide sequence as shown in SEQ ID NO:2: V-142; T-143; Q-144; D-145; C-146; L-147; Q-148; L-149; I-150; A-151; D-152; S-153; E-154; T-155; P-156; T-157; I-158; Q-159; and K-160.

[0354] Recombinant DNA technology known to those skilled in the art (see, for instance, DNA shuffling *supra*) can be used to create novel mutant proteins or muteins including single or multiple amino acid substitutions, deletions, additions or fusion proteins. Such modified polypeptides can show, e.g., enhanced activity or increased

stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions.

[0355] Thus, the invention also encompasses Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV derivatives and analogs that have one or more amino acid residues deleted, added, or substituted to generate Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides that are better suited for expression, scale up, etc., in the host cells chosen. For example, cysteine residues can be deleted or substituted with another amino acid residue in order to eliminate disulfide bridges; N-linked glycosylation sites can be altered or eliminated to achieve, for example, expression of a homogeneous product that is more easily recovered and purified from yeast hosts which are known to hyperglycosylate N-linked sites. To this end, a variety of amino acid substitutions at one or both of the first or third amino acid positions on any one or more of the glycosylation recognition sequences in the Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides of the invention, and/or an amino acid deletion at the second position of any one or more such recognition sequences will prevent glycosylation of the Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV at the modified tripeptide sequence (see, e.g., Miyajimo et al., EMBO J 5(6):1193-1197). By way of non-limiting example, mutation of the serine at position 244 to alanine either singly or in combination with mutation of the asparagine at position 242 to glutamine abolishes glycosylation of the mature soluble form of Neutrokin- $\alpha$  (amino acids 134-285) of SEQ ID NO:2) when expressed in the yeast *Pichia pastoris*. A mutant Neutrokin- $\alpha$  polypeptide in which only the asparagine at position 242 is mutated to glutamine, is still glycosylated when expressed in *Pichia pastoris*. In this mutant, the glycosylation event may be due to the activation or unmasking of an O-linked glycosylation site at serine 244. Similar mutations affecting glycosylation could also be made in Neutrokin  $\alpha$ -SV polypeptide, i.e., asparagine-223 to glutamine and/or serine-224 to alanine of SEQ ID NO:19.

[0356] Additionally, one or more of the amino acid residues of the polypeptides of the invention (e.g., arginine and lysine residues) may be deleted or substituted with another residue to eliminate undesired processing by proteases such as, for example, furins or kexins. One possible result of such a mutation is that Neutrokin- $\alpha$  polypeptide of the invention is not cleaved and released from the cell surface.

[0357] In a specific embodiment, Lys-132 and/or Arg-133 of the Neutrokin- $\alpha$  sequence shown in SEQ ID NO:2 is mutated to another amino acid residue, or deleted altogether, to prevent or diminish release of the soluble form of Neutrokin- $\alpha$  from cells expressing Neutrokin- $\alpha$ . In a more specific embodiment, Lys-132 of the Neutrokin- $\alpha$  sequence shown in SEQ ID NO:2 is mutated to Ala-132. In another, nonexclusive specific embodiment, Arg-133 of the Neutrokin- $\alpha$  sequence shown in SEQ ID NO:2 is mutated to Ala-133. These mutated proteins, and/or polynucleotides encoding these proteins have uses such as, for example, in ex vivo therapy or gene therapy, to engineer cells expressing a Neutrokin- $\alpha$  polypeptide that is retained on the surface of the engineered cells.

[0358] In a specific embodiment, Cys-146 of the Neutrokin- $\alpha$  sequence shown in SEQ ID NO:2 is mutated to another amino acid residue, or deleted altogether, for example, to aid preventing or diminishing oligomerization of the mutant Neutrokin- $\alpha$  polypeptide when expressed in an expression system (essentially as described in Example 1). In a specific embodiment, Cys-146 is replaced with a serine amino acid residue. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0359] In another specific embodiment, Cys-232 of the Neutrokin- $\alpha$  sequence shown in SEQ ID NO:2 is mutated to another amino acid residue, or deleted altogether, for example, to aid preventing or diminishing oligomerization of the mutant Neutrokin- $\alpha$  polypeptide when expressed in an expression system (essentially as described in Example 1). In a specific embodiment, Cys-232 is replaced with a serine amino acid residue. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0360] In yet another specific embodiment, Cys-245 of the Neutrokin- $\alpha$  sequence shown in SEQ ID NO:2 is mutated to another amino acid residue, or deleted altogether, for example, to aid preventing or diminishing oligomerization of the mutant Neutrokin- $\alpha$  polypeptide when expressed in an expression system (essentially as described in Example 1). In a specific embodiment, Cys-245 is replaced with a serine amino acid residue. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0361] The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of the



Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides can be substantially purified by the one-step method described in Smith and Johnson, *Gene* 67:31-40 (1988).

**[0362]** The polypeptides of the present invention include the complete polypeptide encoded by the deposited cDNA (ATCC Deposit No. 97768) including the intracellular, transmembrane and extracellular domains of the polypeptide encoded by the deposited cDNA, the mature soluble polypeptide encoded by the deposited cDNA, the extracellular domain minus the intracellular and transmembrane domains of the protein, the complete polypeptide of Figures 1A and 1B (amino acid residues 1-285 of SEQ ID NO:2), the mature soluble polypeptide of Figures 1A and 1B (amino acids 134-285 of SEQ ID NO:2), the extracellular domain of Figures 1A and 1B (amino acid residues 73-285 of SEQ ID NO:2) minus the intracellular and transmembrane domains, as well as polypeptides which have at least 80%, 85%, 90% similarity, more preferably at least 95% similarity, and still more preferably at least 96%, 97%, 98% or 99% similarity to those described above. Polynucleotides encoding these polypeptides are also encompassed by the invention.

**[0363]** The polypeptides of the present invention also include the complete polypeptide encoded by the deposited cDNA including the intracellular, transmembrane and extracellular domains of the polypeptide encoded by the deposited cDNA (ATCC Deposit No. 203518), the mature soluble polypeptide encoded by the deposited cDNA, the extracellular domain minus the intracellular and transmembrane domains of the protein, the complete polypeptide of Figures 5A and 5B (amino acid residues 1-266 of SEQ ID NO:19), the mature soluble polypeptide of Figures 5A and 5B (amino acid residues 134-266 of SEQ ID NO:19), the extracellular domain of Figures 5A and 5B (amino acid residues 73-266 of SEQ ID NO:19) minus the intracellular and transmembrane domains, as well as polypeptides which have at least 80%, 85%, 90% similarity, more preferably at least 95% similarity, and still more preferably at least 96%, 97%, 98% or 99% similarity to those described above. Polynucleotides encoding these polypeptides are also encompassed by the invention.

**[0364]** Further polypeptides of the present invention include polypeptides at least 80%, or at least 85% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to the polypeptide encoded by the deposited cDNA (ATCC Deposit No. 97768) or to the polypeptide of Figures 1A and 1B (SEQ ID NO:2), and also include portions of such polypeptides with at least 30 amino

acids and more preferably at least 50 amino acids. Polynucleotides encoding these polypeptides are also encompassed by the invention.

**[0365]** Further polypeptides of the present invention include polypeptides at least 80%, or at least 85% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to the polypeptide encoded by the deposited cDNA (ATCC Deposit No. 203518) or to the polypeptide of Figures 5A and 5B (SEQ ID NO:19), and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids. Polynucleotides encoding these polypeptides are also encompassed by the invention.

**[0366]** By "% similarity" for two polypeptides is intended a similarity score produced by comparing the amino acid sequences of the two polypeptides using the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711) and the default settings for determining similarity. Bestfit uses the local homology algorithm of Smith and Waterman (Advances in Applied Mathematics 2:482-489, 1981) to find the best segment of similarity between two sequences.

**[0367]** By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of a Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

**[0368]** As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence

shown in Figures 1A and 1B (SEQ ID NO:2), the amino acid sequence encoded by the deposited cDNA clone HNEDU15 (ATCC Accession No. 97768), or fragments thereof, or, for instance, to the amino acid sequence shown in Figures 5A and 5B (SEQ ID NO:19), the amino acid sequence encoded by the deposited cDNA clone HDPMC52 (ATCC Accession No. 203518), or fragments thereof, can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

**[0369]** In a specific embodiment, the identity between a reference (query) sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, is determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245 (1990)). Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter. According to this embodiment, if the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction is made to the results to take into consideration the fact that the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. A determination of whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified

parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of this embodiment. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence. For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are made for the purposes of this embodiment.

**[0370]** The polypeptides of the present invention have uses that include, but are not limited to, as a molecular weight marker on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those skilled in the art. Additionally, as described in detail below, the polypeptides of the present invention have uses that include, but are not limited to, raising polyclonal and monoclonal antibodies, which are useful in assays for detecting Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide expression as described below or as agonists and antagonists capable of enhancing or inhibiting Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV function. The polypeptides of the invention also have therapeutic uses as described herein. Further, such polypeptides can be used in the yeast two-hybrid system to "capture" Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV binding proteins which are also candidate agonists and antagonists according to the

present invention. The yeast two hybrid system is described in Fields and Song, *Nature* 340:245-246 (1989).

### ***Transgenics and "knock-outs"***

[0371] The polypeptides of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, *e.g.*, baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment, techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy protocol.

[0372] Any technique known in the art may be used to introduce the transgene (*i.e.*, polynucleotides of the invention) into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson, *et al.*, *Appl. Microbiol. Biotechnol.* 40:691-698 (1994); Carver *et al.*, *Biotechnology* (NY) 11:1263-1270 (1993); Wright *et al.*, *Biotechnology* (NY) 9:830-834 (1991); and Hoppe *et al.*, U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten *et al.*, *Proc. Natl. Acad. Sci., USA* 82:6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson *et al.*, *Cell* 56:313-321 (1989)); electroporation of cells or embryos (Lo, 1983, *Mol Cell. Biol.* 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, *e.g.*, Ulmer *et al.*, *Science* 259:1745 (1993); introducing nucleic acid constructs into embryonic pluripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (Lavitrano *et al.*, *Cell* 57:717-723 (1989); *etc.* For a review of such techniques, see Gordon, "Transgenic Animals," *Intl. Rev. Cytol.* 115:171-229 (1989), which is incorporated by reference herein in its entirety. See also, U.S. Patent No. 5,464,764 (Capecchi, *et al.*, Positive-Negative Selection Methods and Vectors); U.S. Patent No. 5,631,153 (Capecchi, *et al.*, Cells and Non-Human Organisms Containing Predetermined Genomic Modifications and Positive-Negative Selection Methods and Vectors for Making Same); U.S. Patent No. 4,736,866 (Leder, *et al.*, Transgenic Non-Human Animals); and U.S. Patent No. 4,873,191 (Wagner, *et al.*, Genetic Transformation of Zygotes); each of which is hereby incorporated by reference in its entirety.

[0373] Any technique known in the art may be used to produce transgenic clones containing polynucleotides of the invention, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campell et al., Nature 380:64-66 (1996); Wilmut et al., Nature 385:810-813 (1997)).

[0374] The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, *i.e.*, mosaic or chimeric animals. The transgene may be integrated as a single transgene or as multiple copies such as in concatamers, *e.g.*, head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., Proc. Natl. Acad. Sci. USA 89:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., Science 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. In addition to expressing the polypeptide of the present invention in a ubiquitous or tissue specific manner in transgenic animals, it would also be routine for one skilled in the art to generate constructs which regulate expression of the polypeptide by a variety of other means (for example, developmentally or chemically regulated expression).

[0375] Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using

techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, reverse transcriptase-PCR (rt-PCR); and TaqMan PCR. Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

[0376] Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; breeding to place the transgene on a distinct background that is appropriate for an experimental model of interest; and breeding of transgenic animals to other animals bearing a distinct transgene or knockout mutation.

[0377] Transgenic and “knock-out” animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides, studying conditions and/or disorders associated with aberrant Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

[0378] In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (e.g., knockouts) are administered to a patient *in vivo*. Such cells may be obtained from the patient (i.e., animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence

associated with the polypeptides of the invention, e.g., by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally.

[0379] Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by reference herein in its entirety).

[0380] When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

### ***Antibodies***

[0381] Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment, or variant of SEQ ID NO:2 and/or SEQ ID NO:19, and/or an epitope, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding). In specific embodiments, antibodies of the invention bind homomeric, especially homotrimeric, Neutrokin- $\alpha$  polypeptides. In other specific embodiments, antibodies of the invention bind heteromeric, especially heterotrimeric, Neutrokin- $\alpha$  polypeptides such as a heterotrimer containing two Neutrokin- $\alpha$



polypeptides and one APRIL polypeptide (e.g., SEQ ID NO:20 or SEQ ID NO:47) or a heterotrimer containing one Neutrokin-alpha polypeptide and two APRIL polypeptides.

**[0382]** In particularly preferred embodiments, the antibodies of the invention bind homomeric, especially homotrimeric, Neutrokin-alpha polypeptides, wherein the individual protein components of the multimers consist of the mature form of Neutrokin alpha (e.g., amino acids residues 134-285 of SEQ ID NO:2, or amino acids residues 134-266 of SEQ ID NO:19.) In other specific embodiments, antibodies of the invention bind heteromeric, especially heterotrimeric, Neutrokin-alpha polypeptides such as a heterotrimer containing two Neutrokin-alpha polypeptides and one APRIL polypeptide or a heterotrimer containing one Neutrokin-alpha polypeptide and two APRIL polypeptides, and wherein the individual protein components of the Neutrokin-alpha heteromer consist of the mature extracellular soluble portion of either Neutrokin-alpha or (e.g., amino acids residues 134-285 of SEQ ID NO:2, or amino acids residues 134-266 of SEQ ID NO:19) or the mature extracellular soluble portion APRIL (e.g., amino acid residues 105-250 of SEQ ID NO:47).

**[0383]** In specific embodiments, the antibodies of the invention bind conformational epitopes of a Neutrokin-alpha and/or Neutrokin-alphaSV monomeric protein. In specific embodiments, the antibodies of the invention bind conformational epitopes of a Neutrokin-alpha and/or Neutrokin-alphaSV multimeric, especially trimeric, protein. In other embodiments, antibodies of the invention bind conformational epitopes that arise from the juxtaposition of Neutrokin-alpha and/or Neutrokin alpha SV with a heterologous polypeptide, such as might be present when Neutrokin-alpha or Neutrokin-alpha SV forms heterotrimers (e.g., with APRIL polypeptides (e.g., SEQ ID NO:20 or SEQ ID NO:47)), or in fusion proteins between Neutrokin alpha and a heterologous polypeptide.

**[0384]** Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen

binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. . In preferred embodiments, the immunoglobulin is an IgG1 or an IgG4 isotype. Immunoglobulins may have both a heavy and light chain. An array of IgG, IgE, IgM, IgD, IgA, and IgY heavy chains may be paired with a light chain of the kappa or lambda forms.

**[0385]** Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')<sub>2</sub>, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, sheep rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

**[0386]** The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

**[0387]** Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and Figures. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

**[0388]** In specific embodiments, antibodies of the invention bind to polypeptides comprising Phe-115 to Leu-147, Ile-150 to Tyr-163, Ser-171 to Phe-194, Glu-223 to Tyr-246, and Ser-271 to Phe-278 of the amino acid sequence of SEQ ID NO:2. In another specific embodiment, antibodies of the invention bind to polypeptides consisting of Phe-115 to Leu-147, Ile-150 to Tyr-163, Ser-171 to Phe-194, Glu-223 to Tyr-246, and Ser-271 to Phe-278 of the amino acid sequence of SEQ ID NO:2. In a preferred embodiment, antibodies of the invention bind to a polypeptide comprising Glu-223 to Tyr-246 of SEQ ID NO:2. In another preferred embodiment, antibodies of the invention bind to a polypeptide consisting of Glu-223 to Tyr-246 of SEQ ID NO:2. In a more preferred embodiment, antibodies of the invention bind to a polypeptide consisting of Phe-230 to Asn-242 of SEQ ID NO:2. In further preferred, nonexclusive embodiments, the antibodies of the invention inhibit one or more biological activities of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides of the invention through specific binding. In more preferred embodiments, the antibody of the invention inhibits Neutrokin-alpha and/or Neutrokin-alphaSV-mediated B cell proliferation.

**[0389]** Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In a specific embodiment, antibodies of the present invention cross react with APRIL (e.g., SEQ ID NO:20 or SEQ ID NO:47; PCT International Publication Number WO97/33902; GenBank Accession No. AF046888

(nucleotide) and AAC6132 (protein); J. Exp. Med. 188(6):1185-1190). In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. In specific embodiments, antibodies of the invention bind Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides, or fragments or variants thereof, with a dissociation constant or  $K_D$  of less than or equal to  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M,  $5 \times 10^{-5}$  M, or  $10^{-5}$  M. More preferably, antibodies of the invention bind Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides or fragments or variants thereof with a dissociation constant or  $K_D$  less than or equal to  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M, or  $10^{-8}$  M. Even more preferably, antibodies of the invention bind Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides or fragments or variants thereof with a dissociation constant or  $K_D$  less than or equal to  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, or  $10^{-15}$  M. The invention encompasses antibodies that bind Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptides with a dissociation constant or  $K_D$  that is within any one of the ranges that are between each of the individual recited values.

**[0390]** The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at

least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

[0391] Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferably, antibodies of the present invention bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described supra). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

[0392] The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res. 58(16):3668-3678 (1998); Harrop et al., J. Immunol.

161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

**[0393]** Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both *in vitro* and *in vivo* diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

**[0394]** As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

**[0395]** The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage,

acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

[0396] The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of-interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

[0397] Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

[0398] A "monoclonal antibody" may comprise, or alternatively consist of, two proteins, i.e., a heavy and a light chain.

[0399] Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art and are discussed in detail in the Examples (e.g., Example 9). In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the

mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well-known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

**[0400]** Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

**[0401]** Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')<sub>2</sub> fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). F(ab')<sub>2</sub> fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

**[0402]** For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include



those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

**[0403]** As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')<sub>2</sub> fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., BioTechniques 12(6):864-869 (1992); and Sawai et al., AJRI 34:26-34 (1995); and Better et al., Science 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

**[0404]** Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., Methods in Enzymology 203:46-88 (1991); Shu et al., PNAS 90:7995-7999 (1993); and Skerra et al., Science 240:1038-1040 (1988). For some uses, including *in vivo* use of antibodies in humans and *in vitro* detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies et al., (1989) J. Immunol. Methods 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions

(CDRs) from the non-human species and a framework region from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., *Nature* 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, *Molecular Immunology* 28(4/5):489-498 (1991); Studnicka et al., *Protein Engineering* 7(6):805-814 (1994); Roguska et al., *PNAS* 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

**[0405]** Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

**[0406]** Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous

antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, *Int. Rev. Immunol.* 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

**[0407]** Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., *Bio/technology* 12:899-903 (1988)).

**[0408]** Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, *FASEB J.* 7(5):437-444; (1989) and Nissinoff, *J. Immunol.* 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-

idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

### **Polynucleotides Encoding Antibodies**

**[0409]** The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined *supra*, to polynucleotides that encode an antibody, preferably, that specifically binds to a polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:2. In another preferred embodiment, the antibody binds specifically to a polypeptide having the amino acid sequence of SEQ ID NO:19. In another preferred embodiment, the antibody binds specifically to a polypeptide having the amino acid sequence of SEQ ID NO:23. In another preferred embodiment, the antibody binds specifically to a polypeptide having the amino acid sequence of SEQ ID NO:28. In another preferred embodiment, the antibody binds specifically to a polypeptide having the amino acid sequence of SEQ ID NO:30. In another preferred embodiment, the antibody binds specifically to a polypeptide having the amino acid sequence of SEQ ID NO:39. In another preferred embodiment, the antibody binds specifically to a polypeptide having the amino acid sequence of SEQ ID NO:40. In another embodiment, the antibody binds specifically to a polypeptide having the amino acid sequence of SEQ ID NO:41. In another embodiment, the antibody binds specifically to a polypeptide having the amino acid sequence of SEQ ID NO:42. In another embodiment, the antibody binds specifically to a polypeptide having the amino acid sequence of SEQ ID NO:43. In another embodiment, the antibody binds specifically to a polypeptide having the amino acid sequence of SEQ ID NO:44.

**[0410]** The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., *BioTechniques* 17:242 (1994)), which, briefly, involves the synthesis of

overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[0411] Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A<sup>+</sup> RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

[0412] Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

[0413] In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well known in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described

*supra*. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed *supra*, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

[0414] In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described *supra*, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

[0415] Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423- 42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* may also be used (Skerra et al., Science 242:1038- 1041 (1988)).

### **Methods of Producing Antibodies**

[0416] The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

[0417] Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

[0418] The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

[0419] A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate

nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., Gene 45:101 (1986); Cockett et al., Bio/Technology 8:2 (1990)).

**[0420]** In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., EMBO J. 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, Nucleic Acids Res. 13:3101-3109 (1985); Van Heeke & Schuster, J. Biol. Chem. 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified



from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[0421] In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

[0422] In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (E.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

[0423] In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed.

To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

**[0424]** For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

**[0425]** A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson,

Ann. Rev. Biochem. 62:191-217 (1993); May, 1993, TIB TECH 11(5):155-215); and hygromycin, which confers resistance to hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

[0426] The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

[0427] The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

[0428] Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition,

the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

[0429] The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalent and non-covalent conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either *in vitro* or *in vivo*, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in *in vitro* immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., *supra*, and PCT publication WO 93/21232; EP 439,095; Naramura et al., *Immunol. Lett.* 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., *PNAS* 89:1428-1432 (1992); Fell et al., *J. Immunol.* 146:2446-2452(1991), which are incorporated by reference in their entireties.

[0430] The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications

WO 96/04388; WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337-11341(1992) (said references incorporated by reference in their entireties).

**[0431]** As discussed, *supra*, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of SEQ ID NO:2 may be fused or conjugated to the above antibody portions to increase the *in vivo* half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to SEQ ID NO:2 may be fused or conjugated to the above antibody portions to facilitate purification. Also as discussed, *supra*, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of SEQ ID NO:19 may be fused or conjugated to the above antibody portions to increase the *in vivo* half life of the polypeptides or for use in immunoassays using methods known in the art. Moreover, the polypeptides corresponding to SEQ ID NO:19 may be fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Traunecker et al., Nature 331:84-86 (1988). The polypeptides of the present invention fused or conjugated to an antibody having disulfide- linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A 232,262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, Bennett et al., J. Molecular Recognition 8:52-58 (1995); Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).

**[0432]** Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred

embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the "flag" tag.

[0433] The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include iodine ( $^{131}\text{I}$ ,  $^{125}\text{I}$ ,  $^{123}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{115\text{m}}\text{In}$ ,  $^{113\text{m}}\text{In}$ ,  $^{112}\text{In}$ ,  $^{111}\text{In}$ ), and technetium ( $^{99\text{m}}\text{Tc}$ ,  $^{99}\text{Tc}$ ), thallium ( $^{201}\text{Tl}$ ), gallium ( $^{68}\text{Ga}$ ,  $^{67}\text{Ga}$ ), palladium ( $^{103}\text{Pd}$ ), molybdenum ( $^{99}\text{Mo}$ ), xenon ( $^{133}\text{Xe}$ ), fluorine ( $^{18}\text{F}$ ),  $^{153}\text{Sm}$ ,  $^{177}\text{Lu}$ ,  $^{159}\text{Gd}$ ,  $^{149}\text{Pm}$ ,  $^{140}\text{La}$ ,  $^{175}\text{Yb}$ ,  $^{166}\text{Ho}$ ,  $^{90}\text{Y}$ ,  $^{47}\text{Sc}$ ,  $^{186}\text{Re}$ ,

<sup>188</sup>Re, <sup>142</sup>Pr, <sup>105</sup>Rh, <sup>97</sup>Ru, <sup>68</sup>Ge, <sup>57</sup>Co, <sup>65</sup>Zn, <sup>85</sup>Sr, <sup>32</sup>P, <sup>153</sup>Gd, <sup>169</sup>Yb, <sup>51</sup>Cr, <sup>54</sup>Mn, <sup>75</sup>Se, <sup>113</sup>Sn, and <sup>117</sup>Tin.

**[0434]** Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, <sup>213</sup>Bi. In specific embodiments, antibodies of the invention are attached to macrocyclic chelators useful for conjugating radiometal ions, including but not limited to, <sup>111</sup>In, <sup>177</sup>Lu, <sup>90</sup>Y, <sup>166</sup>Ho, and <sup>153</sup>Sm, to polypeptides. In preferred embodiments, the radiometal ion associated with the macrocyclic chelators attached to antibodies of the invention is <sup>111</sup>In. In preferred embodiments, the radiometal ion associated with the macrocyclic chelators attached to antibodies of the invention is <sup>90</sup>Y. In specific embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA). In other specific embodiments, the DOTA is attached to the Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide of the invention via a linker molecule. Examples of linker molecules useful for conjugating DOTA to a polypeptide are commonly known in the art - see, for example, DeNardo et al., *Clin Cancer Res.* 4(10):2483-90 (1998); Peterson et al., *Bioconjug. Chem.* 10(4):553-7 (1999); and Zimmerman et al, *Nucl. Med. Biol.* 26(8):943-50 (1999) which are hereby incorporated by reference in their entirety. In addition, U.S. Patents 5,652,361 and 5,756,065, which disclose chelating agents that may be conjugated to antibodies, and methods for making and using them, are hereby incorporated by reference in their entireties.

**[0435]** A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells and includes such molecules as small molecule toxins and enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide (VP-16), tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide,

busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine), improsulfan, piposulfan, benzodopa, carboquone, meturedopa, uredopa, altretamine, triethylenemelamine, triethylenephosphoramidate, triethylenethiophosphoramidate, trimethylolomelamine, chlornaphazine, cholophosphamide, estramustine, ifosfamide, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard, chlorozotocin, fotemustine, nimustine, ranimustine, aclacinomysins, azaserine, cactinomycin, calichearnicin, carabycin, carminomycin, carzinophilin, chromomycins, detorubicin, 6-diazo-5-oxo-L-norleucine, epirubicin, esorubicin, idarubicin, marcellomycin, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, quelamycin, rodorubicin, streptonigrin, tubercidin, ubenimex, zinostatin, zorubicin, denopterin, pteropterin, trimetrexate, fludarabine, thiamiprine, ancitabine, azacitidine, 6-azauridine, carmofur, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU, calusterone, dromostanolone propionate, epitio stanol, mepitio stanol, testolactone, aminogluthethimide, mitotane, trilostane, frolinic acid, aceglatone, aldophosphamide glycoside, aminolevulinic acid, amsacrine, bestabucil, bisantrene, edatraxate, defofamine, dernecolcine, diaziquone, elfornithine, elliptinium acetate, etoglucid, gallium nitrate, hydroxyurea, lentinan, lonidamine, mitoguazone, mopidamol, nitracrine, pentostatin, phenamet, pirarubicin, podophyllinic acid, 2-ethylhydrazide, procarbazine, PSKO, razoxane, sizofiran, spirogermanium, tenuazonic acid, triaziquone, 2, 2',2''-trichlorotriethylamine, urethan, vindesine, dacarbazine, mannomustine, mitobronitol, mitolactol, pipobroman, gacytosine, arabinoside ("Ara-C"), taxoids, e.g. paclitaxel (TAXOL", Bristol-Myers Squibb Oncology, Princeton, NJ) doxetaxel (TAXOTERE", Rhône-Poulenc Rorer, Antony, France), gemcitabine, ifosfamide, vinorelbine, navelbine, novantrone, teniposide, aminopterin, xeloda, ibandronate, CPT-I 1, topoisomerase inhibitor RFS 2000, difluoromethylornithine (DMFO), retinoic acid, esperamicins, capecitabine, and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4 hydroxytamoxifen, trioxifene,



keoxifene, LY 117018, onapristone, toremifene (Fareston), and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin, and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0436] The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF-alpha, TNF-beta, AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al.*, *Int. Immunol.*, 6:1567-1574 (1994)), VEGI (See, International Publication No. WO 99/23105), CD40 Ligand, a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

[0437] Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

[0438] Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody in Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin *et al.* (eds.), pp. 303-16

(Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* 62:119-58 (1982).

[0439] Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

[0440] An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

#### Immunophenotyping

[0441] The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of the gene of the present invention may be useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison *et al.*, *Cell*, 96:737-49 (1999)).

[0442] These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

#### Assays For Antibody Binding

[0443] The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used, include but are not limited to, competitive and non-competitive assay systems using techniques such as

western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

**[0444]** Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4°C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

**[0445]** Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate

(e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g.,  $^{32}\text{P}$  or  $^{125}\text{I}$ ) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

**[0446]** ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

**[0447]** The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g.,  $^3\text{H}$  or  $^{125}\text{I}$ ) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest conjugated to a labeled compound (e.g.,  $^3\text{H}$  or  $^{125}\text{I}$ ) in the presence of increasing amounts of an unlabeled second antibody.

**Therapeutic Uses**

[0448] The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention and/or a receptor for the polypeptide of the invention (e.g., transmembrane activator and CAML interactor (TACI, GenBank accession number AAC51790), and B-cell maturation antigen (BCMA, GenBank accession number NP\_001183)), including, but not limited to, any one or more of the diseases, disorders, or conditions described herein (e.g., autoimmune diseases, disorders, or conditions associated with such diseases or disorders, including, but not limited to, autoimmune hemolytic anemia (including but not limited to cryoglobulinemia or Coombs positive anemia), autoimmune neonatal thrombocytopenia, idiopathic thrombocytopenia purpura, autoimmunocytopenia, autoimmune neutropenia, hemolytic anemia, antiphospholipid syndrome, dermatitis (e.g., atopic dermatitis), allergic encephalomyelitis, myocarditis, relapsing polychondritis, rheumatic heart disease, Multiple Sclerosis, Neuritis, Uveitis Ophthalmia, Polyendocrinopathies, Purpura (e.g., Henloch-Scoenlein purpura), Reiter's Disease, Stiff-Man Syndrome, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, diabetes mellitus (e.g., Type I diabetes mellitus or insulin dependent diabetes mellitus), juvenile onset diabetes, and autoimmune inflammatory eye, autoimmune thyroiditis, hypothyroidism (i.e., Hashimoto's thyroiditis, systemic lupus erythematosus, discoid lupus, Goodpasture's syndrome, Pemphigus, Receptor autoimmunities such as, for example, (a) Graves' Disease, (b) Myasthenia Gravis, and (c) insulin resistance, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, rheumatoid arthritis, scleroderma with anti-collagen antibodies, mixed connective tissue disease, polymyositis/dermatomyositis, pernicious anemia (Addison's disease), idiopathic Addison's disease, infertility, glomerulonephritis such as primary glomerulonephritis, IgA

glomerulonephritis, and IgA nephropathy, bullous pemphigoid, Sjogren's syndrome, diabetes mellitus, and adrenergic drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis), gluten sensitive enteropathy, dense deposit disease, chronic active hepatitis, primary biliary cirrhosis, other endocrine gland failure, vitiligo, vasculitis, post-MI, cardiomyopathy syndrome, urticaria, atopic dermatitis, asthma, inflammatory myopathies, and other inflammatory, granulomatous, degenerative, and atrophic disorders) and other disorders such as inflammatory skin diseases including psoriasis and sclerosis, responses associated with inflammatory bowel disease (such as Crohn's disease and ulcerative colitis), respiratory distress syndrome (including adult respiratory distress syndrome, ARDS), meningitis, encephalitis, colitis, allergic conditions such as eczema and other conditions involving infiltration of T cells and chronic inflammatory responses, atherosclerosis, leukocyte adhesion deficiency, Reynaud's syndrome, and immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes typically found in tuberculosis, sarcoidosis, granulomatosis and diseases involving leukocyte diapedesis, central nervous system (CNS) inflammatory disorder, multiple organ injury syndrome, antigen-antibody complex mediated diseases, anti-glomerular basement membrane disease, Lambert-Eaton myasthenic syndrome, Behcet disease, giant cell arteritis, immune complex nephritis, IgA nephropathy, IgM polyneuropathies or autoimmune thrombocytopenia etc.

[0449] In a specific embodiment, antibodies of the invention are used to treat, inhibit, prognose, diagnose or prevent rheumatoid arthritis. In a specific embodiment, antibodies of the invention are used to treat, inhibit, prognose, diagnose or prevent advanced rheumatoid arthritis.

[0450] In another specific embodiment, antibodies of the invention are used to treat, inhibit, prognose, diagnose or prevent systemic lupus erythematosus.

[0451] For example, an antibody, or antibodies, of the present invention are used to treat patients with clinical diagnosis of rheumatoid arthritis (RA). The patient treated will not have a B cell malignancy. Moreover, the patient is optionally further treated with any one or more agents employed for treating RA such as salicylate; nonsteroidal anti-inflammatory drugs such as indomethacin, phenylbutazone, phenylacetic acid derivatives (*e.g.* ibuprofen and fenoprofen), naphthalene acetic acids (naproxen), pyrrolealkanoic acid (tometin), indoleacetic acids (sulindac), halogenated anthranilic acid

(meclofenamate sodium), piroxicam, zomepirac and diflunisal; antimalarials such as chloroquine; gold salts; penicillamine; or immunosuppressive agents such as methotrexate or corticosteroids in dosages known for such drugs or reduced dosages. Preferably however, the patient is only treated with an antibody, or antibodies, of the present invention. Antibodies of the present invention are administered to the RA patient according to a dosing schedule as described *infra*, which may be readily determined by one of ordinary skill in the art. The primary response is determined by the Paulus index (Paulus et al. Arthritis Rheum. 33:477-484 (1990)), *i.e.* improvement in morning stiffness, number of painful and inflamed joints, erythrocyte sedimentation (ESR), and at least a 2-point improvement on a 5-point scale of disease severity assessed by patient and by physician. Administration of an antibody, or antibodies, of the present invention will alleviate one or more of the symptoms of RA in the patient treated as described above.

[0452] In a further specific embodiment, antibodies of the invention are used to treat, inhibit, prognose, diagnose or prevent hemolytic anemia. For example, patients diagnosed with autoimmune hemolytic anemia (AIHA), *e.g.*, cryoglobulinemia or Coombs positive anemia, are treated with an antibody, or antibodies, of the present invention. AIHA is an acquired hemolytic anemia due to auto-antibodies that react with the patient's red blood cells. The patient treated will not have a B cell malignancy. Further adjunct therapies (such as glucocorticoids, prednisone, azathioprine, cyclophosphamide, vinca-laden platelets or Danazol) may be combined with the antibody therapy, but preferably the patient is treated with an antibody, or antibodies, of the present invention as a single-agent throughout the course of therapy. Antibodies of the present invention are administered to the hemolytic anemia patient according to a dosing schedule as described *infra*, which may be readily determined by one of ordinary skill in the art. Overall response rate is determined based upon an improvement in blood counts, decreased requirement for transfusions, improved hemoglobin levels and/or a decrease in the evidence of hemolysis as determined by standard chemical parameters. Administration of an antibody, or antibodies of the present invention will improve any one or more of the symptoms of hemolytic anemia in the patient treated as described above. For example, the patient treated as described above will show an increase in hemoglobin and an improvement in chemical parameters of hemolysis or return to normal as measured by serum lactic dehydrogenase and/or bilirubin.

[0453] In another specific embodiment, antibodies of the invention are used to treat, inhibit, prognose, diagnose or prevent adult immune thrombocytopenic purpura. Adult immune thrombocytopenic purpura (ITP) is a relatively rare hematologic disorder that constitutes the most common of the immune-mediated cytopenias. The disease typically presents with severe thrombocytopenia that may be associated with acute hemorrhage in the presence of normal to increased megakaryocytes in the bone marrow. Most patients with ITP have an IgG antibody directed against target antigens on the outer surface of the platelet membrane, resulting in platelet sequestration in the spleen and accelerated reticuloendothelial destruction of platelets (Bussell, J.B. Hematol. Oncol. Clin. North Am. (4):179 (1990)). A number of therapeutic interventions have been shown to be effective in the treatment of ITP. Steroids are generally considered first-line therapy, after which most patients are candidates for intravenous immunoglobulin (IVIG), splenectomy, or other medical therapies including vincristine or immunosuppressive/cytotoxic agents. Up to 80% of patients with ITP initially respond to a course of steroids, but far fewer have complete and lasting remissions. Splenectomy has been recommended as standard second-line therapy for steroid failures, and leads to prolonged remission in nearly 60% of cases yet may result in reduced immunity to infection. Splenectomy is a major surgical procedure that may be associated with substantial morbidity (15%) and mortality (2%). IVIG has also been used as second line medical therapy, although only a small proportion of adult patients with ITP achieve remission. Therapeutic options that would interfere with the production of autoantibodies by activated B cells without the associated morbidities that occur with corticosteroids and/or splenectomy would provide an important treatment approach for a proportion of patients with ITP. Patients with clinical diagnosis of ITP are treated with an antibody, or antibodies of the present invention, optionally in combination with steroid therapy. The patient treated will not have a B cell malignancy. Antibodies of the present invention are administered to the RA patient according to a dosing schedule as described *infra*, which may be readily determined by one of ordinary skill in the art. Overall patient response rate is determined based upon a platelet count determined on two consecutive occasions two weeks apart following treatments as described above. See, George et al. "Idiopathic Thrombocytopenic Purpura: A Practice Guideline Developed by Explicit Methods for The American Society of Hematology", Blood 88:3-40 (1996), expressly incorporated herein by reference.



**[0454]** In other embodiments, antibody agonists of the invention are be used to treat, inhibit or prevent immunodeficiencies, and/or disorders, or conditions associated with immunodeficiencies. Such immunodeficiencies include, but are not limited to, severe combined immunodeficiency (SCID)-X linked, SCID-autosomal, adenosine deaminase deficiency (ADA deficiency), X-linked agammaglobulinemia (XLA), Bruton's disease, congenital agammaglobulinemia, X-linked infantile agammaglobulinemia, acquired agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, transient hypogammaglobulinemia of infancy, unspecified hypogammaglobulinemia, agammaglobulinemia, common variable immunodeficiency (CVID) (acquired), Wiskott-Aldrich Syndrome (WAS), X-linked immunodeficiency with hyper IgM, non X-linked immunodeficiency with hyper IgM, selective IgA deficiency, IgG subclass deficiency (with or without IgA deficiency), antibody deficiency with normal or elevated Igs, immunodeficiency with thymoma, Ig heavy chain deletions, kappa chain deficiency, B cell lymphoproliferative disorder (BLPD), selective IgM immunodeficiency, recessive agammaglobulinemia (Swiss type), reticular dysgenesis, neonatal neutropenia, severe congenital leukopenia, thymic aplasia/aplasia or dysplasia with immunodeficiency, ataxia-telangiectasia, short limbed dwarfism, X-linked lymphoproliferative syndrome (XLP), Nezelof syndrome-combined immunodeficiency with Igs, purine nucleoside phosphorylase deficiency (PNP), MHC Class II deficiency (Bare Lymphocyte Syndrome) and severe combined immunodeficiency.

**[0455]** In another specific embodiment, antibodies of the invention are used to treat, inhibit, prognose, diagnose or prevent CVID, or a subgroup of individuals having CVID.

**[0456]** In another specific embodiment, antibody agonists of the invention are used as an adjuvant to stimulate B cell proliferation, immunoglobulin production, and/or to enhance B cell survival.

**[0457]** The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention and/or a receptor for the polypeptide of the invention (e.g., TACI, BCMA) includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. The antibodies of the invention may also be used to target and kill cells expressing Neutrokin-alpha on their surface and/or cells having Neutrokin-alpha bound to their surface.

Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

[0458] A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

[0459] The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

[0460] The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy, anti-tumor agents, antibiotics, and immunoglobulin). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

[0461] It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention, including fragments thereof. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-5}$  M,  $10^{-5}$  M,  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M,  $10^{-8}$  M,  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, and  $10^{-15}$  M.

**Gene Therapy**

[0462] In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

[0463] Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

[0464] For general reviews of the methods of gene therapy, see Goldspiel et al., *Clinical Pharmacy* 12:488-505 (1993); Wu and Wu, *Biotherapy* 3:87-95 (1991); Tolstoshev, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596 (1993); Mulligan, *Science* 260:926-932 (1993); and Morgan and Anderson, *Ann. Rev. Biochem.* 62:191-217 (1993); May, *TIBTECH* 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); and Kriegler, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY (1990).

[0465] In a preferred embodiment, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, *Proc. Natl. Acad. Sci. USA* 86:8932-8935 (1989); Zijlstra et al., *Nature* 342:435-438 (1989). In specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

[0466] Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids *in vitro*, then transplanted into the patient. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

[0467] In a specific embodiment, the nucleic acid sequences are directly administered *in vivo*, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989)).

[0468] In a specific embodiment, viral vectors that contain nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al.,

Biotherapy 6:291-302 (1994), which describes the use of a retroviral vector to deliver the *mdr1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., *J. Clin. Invest.* 93:644-651 (1994); Kiem et al., *Blood* 83:1467-1473 (1994); Salmons and Gunzberg, *Human Gene Therapy* 4:129-141 (1993); and Grossman and Wilson, *Curr. Opin. in Genetics and Devel.* 3:110-114 (1993).

[0469] Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, *Current Opinion in Genetics and Development* 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., *Human Gene Therapy* 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., *Science* 252:431-434 (1991); Rosenfeld et al., *Cell* 68:143- 155 (1992); Mastrangeli et al., *J. Clin. Invest.* 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., *Gene Therapy* 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

[0470] Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., *Proc. Soc. Exp. Biol. Med.* 204:289-300 (1993); U.S. Patent No. 5,436,146).

[0471] Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

[0472] In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-

mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, Meth. Enzymol. 217:599-618 (1993); Cohen et al., Meth. Enzymol. 217:618-644 (1993); Clin., Pharmac. Ther. 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

[0473] The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

[0474] Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include, but are not limited to, epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

[0475] In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

[0476] In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, Cell 71:973-985 (1992); Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771 (1986)).

[0477] In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

#### **Demonstration of Therapeutic or Prophylactic Activity**

[0478] The compounds or pharmaceutical compositions of the invention are preferably tested *in vitro*, and then *in vivo* for the desired therapeutic or prophylactic activity, prior to use in humans. For example, *in vitro* assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, *in vitro* assays which can be used to determine whether administration of a specific compound is indicated, include *in vitro* cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

#### **Therapeutic and/or Prophylactic Administration and Composition**

[0479] The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical composition of the invention, preferably an antibody of the invention. In a preferred embodiment, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

[0480] Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

[0481] Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0482] In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

[0483] In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)



[0484] In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Press, Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); see also Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J. Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)).

[0485] Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

[0486] In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox- like peptide which is known to enter the nucleus (see e.g., Joliet et al., Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

[0487] The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier"

refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

**[0488]** In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water

for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0489] The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0490] The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[0491] For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

[0492] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

## Diagnosis and Imaging

[0493] Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases and/or disorders associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

[0494] The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

[0495] Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell. Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine ( $^{131}\text{I}$ ,  $^{125}\text{I}$ ,  $^{123}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{115\text{m}}\text{In}$ ,  $^{113\text{m}}\text{In}$ ,  $^{112}\text{In}$ ,  $^{111}\text{In}$ ), and technetium ( $^{99}\text{Tc}$ ,  $^{99\text{m}}\text{Tc}$ ), thallium ( $^{201}\text{Tl}$ ), gallium ( $^{68}\text{Ga}$ ,  $^{67}\text{Ga}$ ), palladium

(<sup>103</sup>Pd), molybdenum (<sup>99</sup>Mo), xenon (<sup>133</sup>Xe), fluorine (<sup>18</sup>F), <sup>153</sup>Sm, <sup>177</sup>Lu, <sup>159</sup>Gd, <sup>149</sup>Pm, <sup>140</sup>La, <sup>175</sup>Yb, <sup>166</sup>Ho, <sup>90</sup>Y, <sup>47</sup>Sc, <sup>186</sup>Re, <sup>188</sup>Re, <sup>142</sup>Pr, <sup>105</sup>Rh, <sup>97</sup>Ru; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[0496] In specific embodiments, antibodies of the invention are attached to macrocyclic chelators useful for conjugating radiometal ions, including but not limited to, <sup>177</sup>Lu, <sup>90</sup>Y, <sup>166</sup>Ho, and <sup>153</sup>Sm, to polypeptides. In a preferred embodiment, the radiometal ion associated with the macrocyclic chelator attached to antibodies of the invention is <sup>111</sup>In. In another preferred embodiment, the radiometal ion associated with the macrocyclic chelator attached to antibodies of the invention is <sup>90</sup>Y. In specific embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA). In other specific embodiments, the DOTA is attached to the Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV polypeptide of the invention via a linker molecule. Examples of linker molecules useful for conjugating DOTA to a polypeptide are commonly known in the art - see, for example, DeNardo et al., Clin Cancer Res. 4(10):2483-90, 1998; Peterson et al., Bioconjug. Chem. 10(4):553-7, 1999; and Zimmerman et al., Nucl. Med. Biol. 26(8):943-50, 1999 which are hereby incorporated by reference in their entirety.

[0497] Techniques known in the art may be applied to label proteins of the invention (including antibodies of the invention). Such techniques include, but are not limited to, the use of bifunctional conjugating agents (see e.g., U.S. Patent Nos. 5,756,065; 5,714,631; 5,696,239; 5,652,361; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274,119; 4,994,560; and 5,808,003; the contents of each of which are hereby incorporated by reference in its entirety) and direct coupling reactions (e.g., Bolton-Hunter, Chloramine-T reaction, and Iodogen®-based labelling).

[0498] One embodiment of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: (a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; (b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be

cleared to background level); (c) determining background level; and (d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system. As described herein, specific embodiments of the invention are directed to the use of the antibodies of the invention to quantitate or qualitate concentrations of cells of B cell lineage or cells of monocytic lineage.

[0499] Also as described herein, antibodies of the invention may be used to treat, diagnose, or prognose an individual having an immunodeficiency. In a specific embodiment, antibodies of the invention are used to treat, diagnose, and/or prognose an individual having common variable immunodeficiency disease (CVID) or a subset of this disease. In another embodiment, antibodies of the invention are used to diagnose, prognose, treat or prevent a disorder characterized by deficient serum immunoglobulin production, recurrent infections, and/or immune system dysfunction.

[0500] Also as described herein, antibodies of the invention may be used to treat, diagnose, or prognose an individual having an autoimmune disease or disorder. In a specific embodiment, antibodies of the invention are used to treat, diagnose, and/or prognose an individual having systemic lupus erythematosus, or a subset of the disease. In another specific embodiment, antibodies of the invention are used to treat, diagnose and/or prognose an individual having rheumatoid arthritis, or a subset of this disease.

[0501] It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of <sup>99</sup>mTc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).